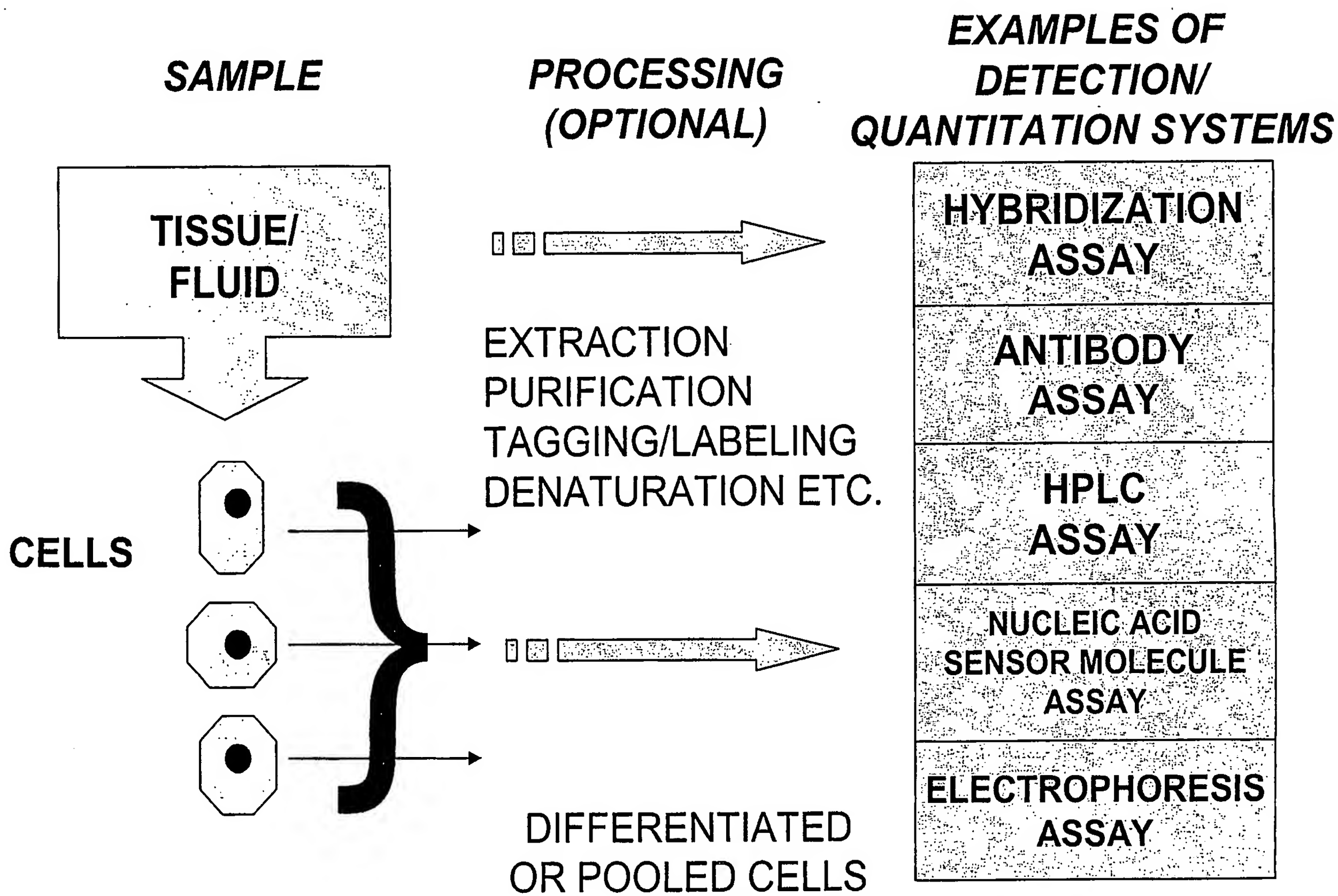
Figure 1A: Detection and Quantitation Of siNA in a Sample



WO 2005/021800
PCT/US2004/026857

Figure 1B: siNA Hybridization Assay

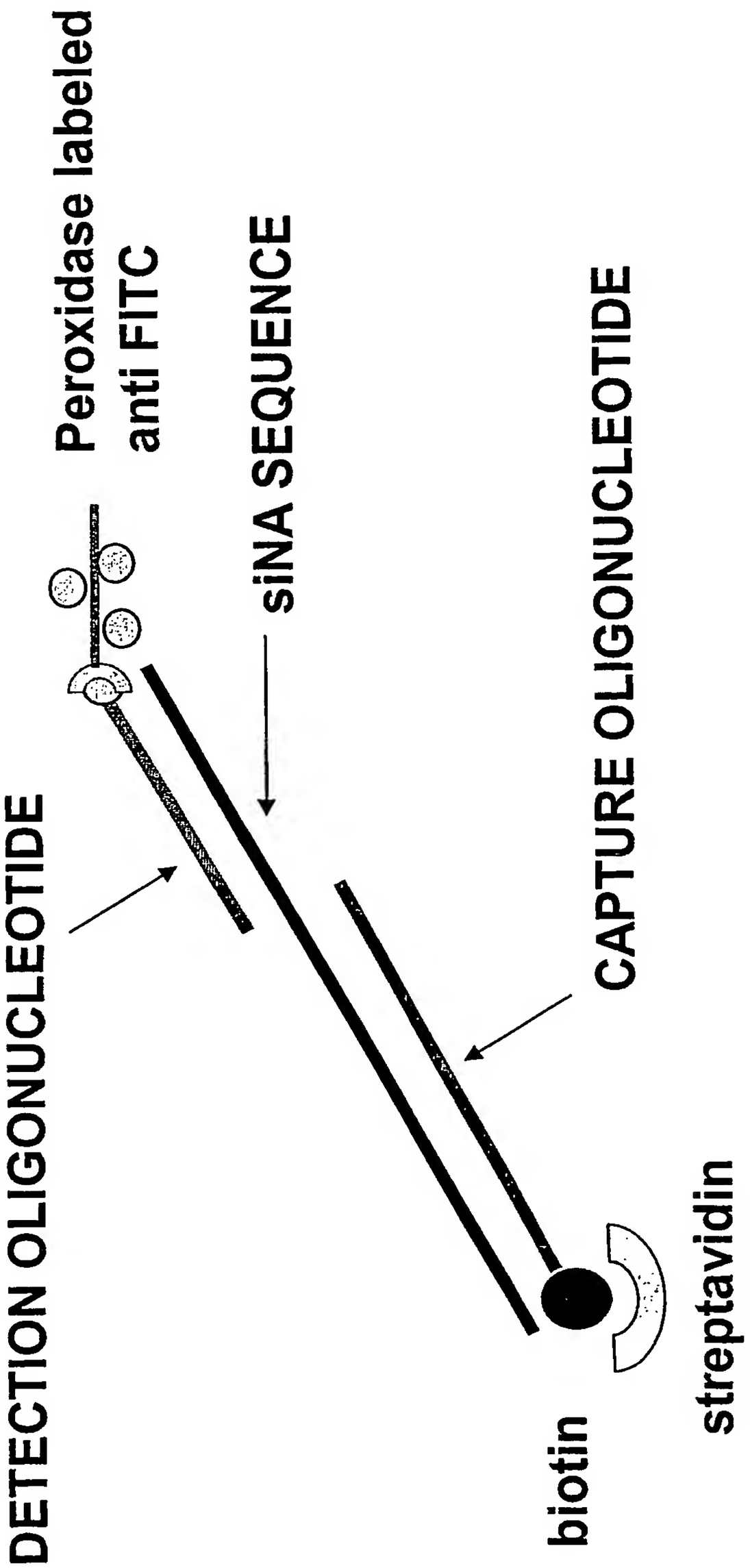
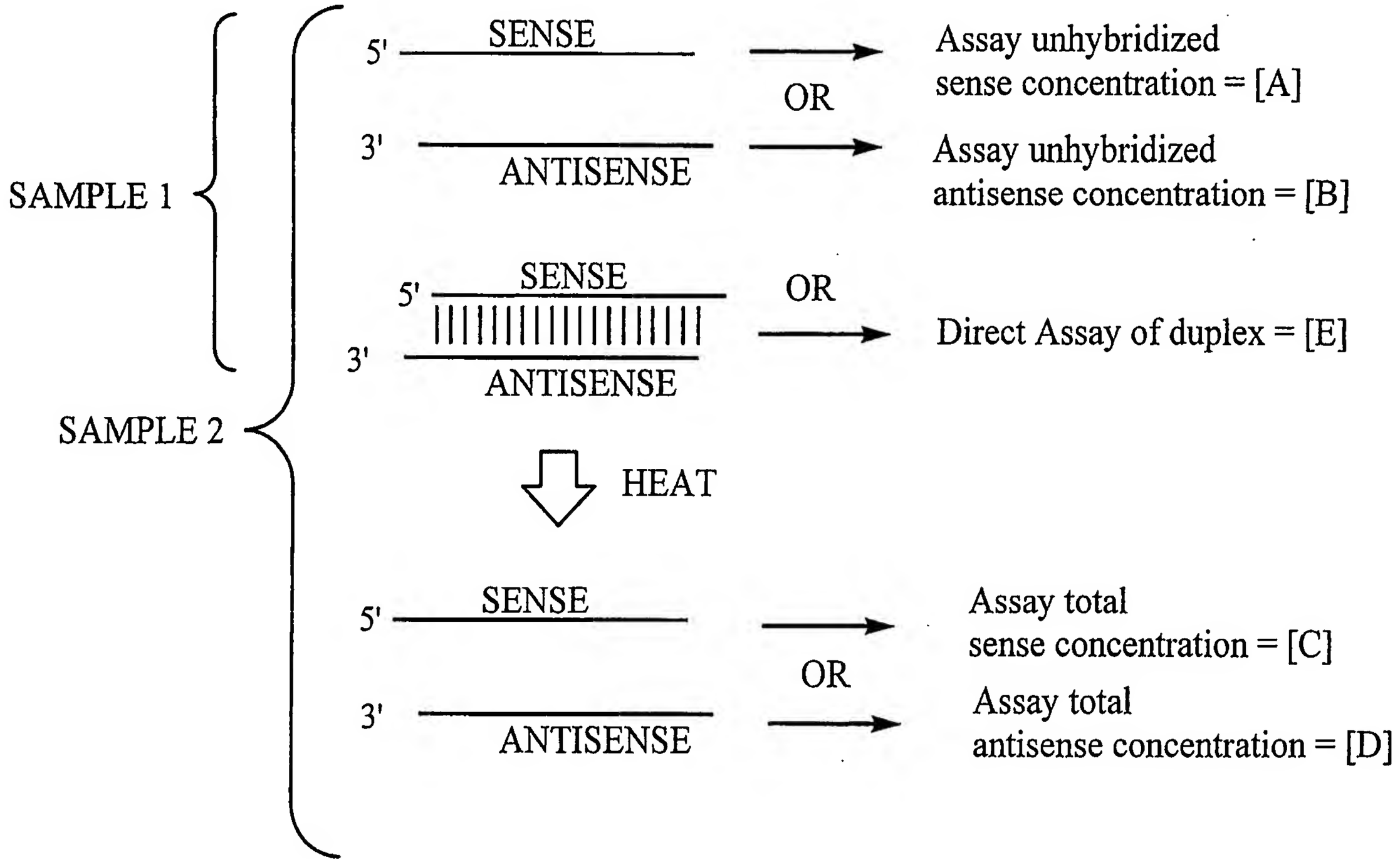


Figure 1C: Principle of siNA detection/quantitation Assays



[C] - [A] = [Duplex] based upon analysis of sense strand
[D] - [B] = [Duplex] based upon analysis of antisense strand
[E] = [Duplex] based upon direct analysis of duplex

PCT/US2004/026857

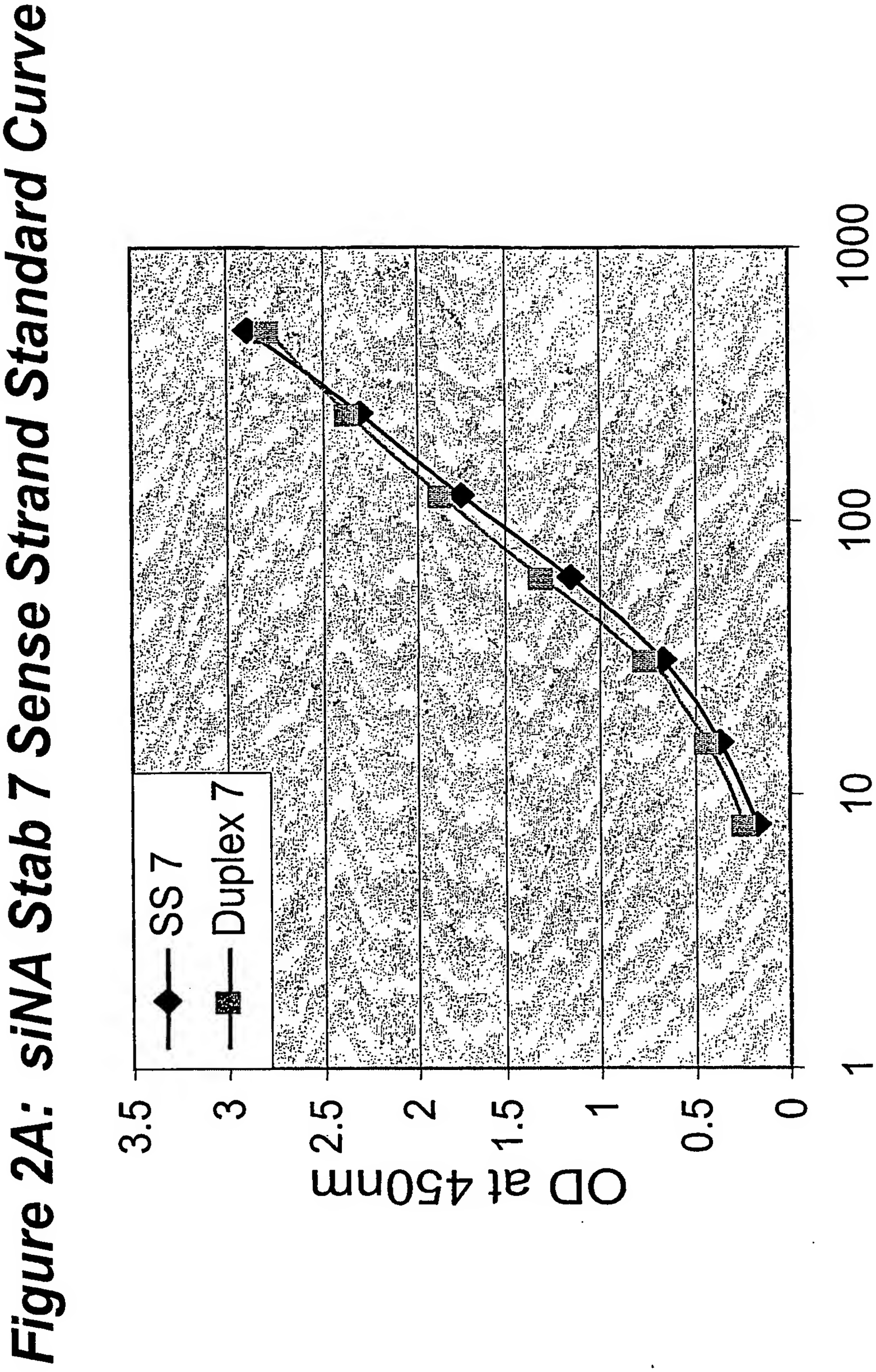

siNA Stab 7 Sense Strand Standard Curve
SS 7
Duplex 7
OD at 450nm
3.5
3
2.5
2
1.5
1
0.5
0
1
10
100
1000
fmols/ml

Figure 2A:

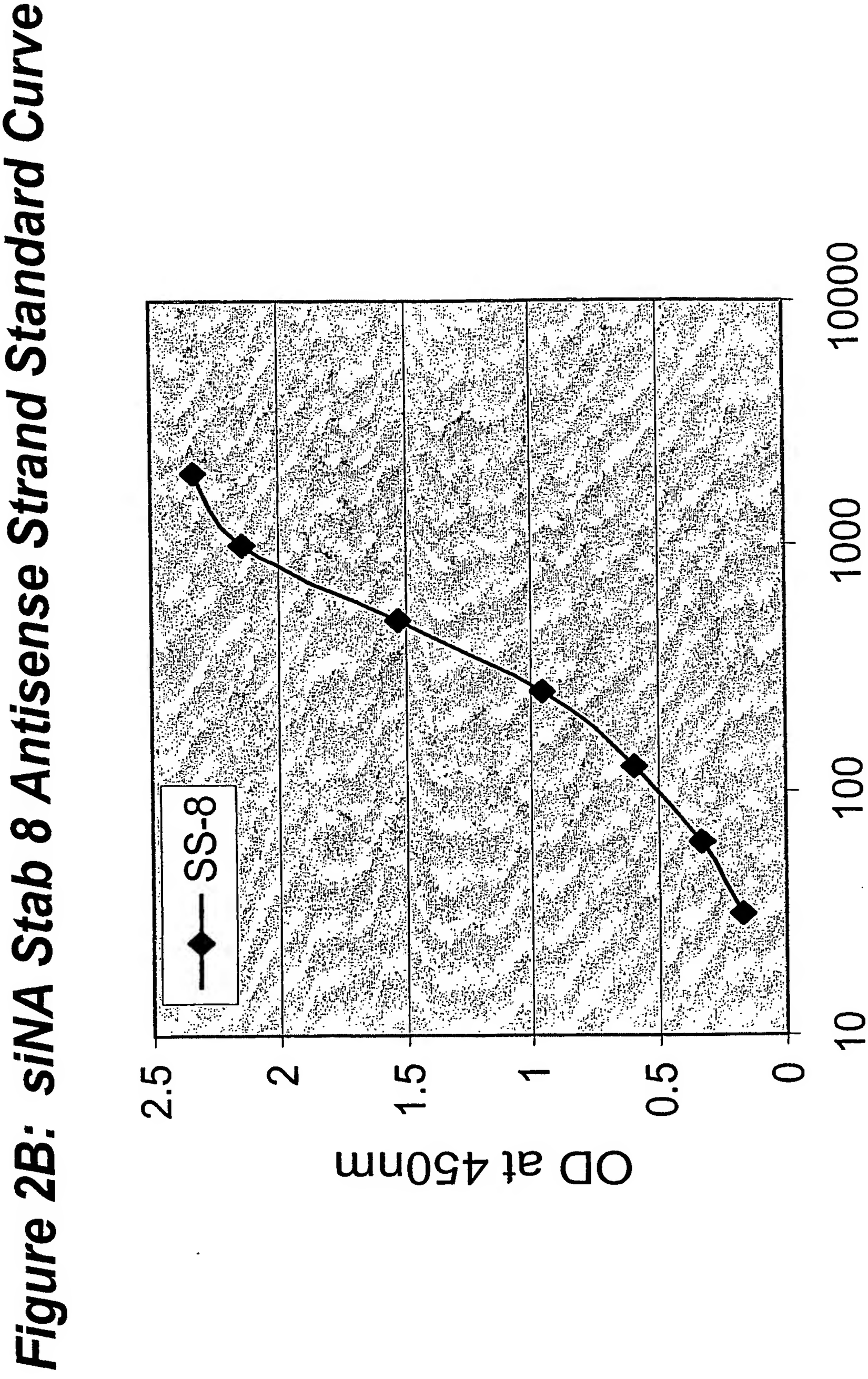


Figure 2B: siRNA Stab 8 Antisense Strand Standard Curve

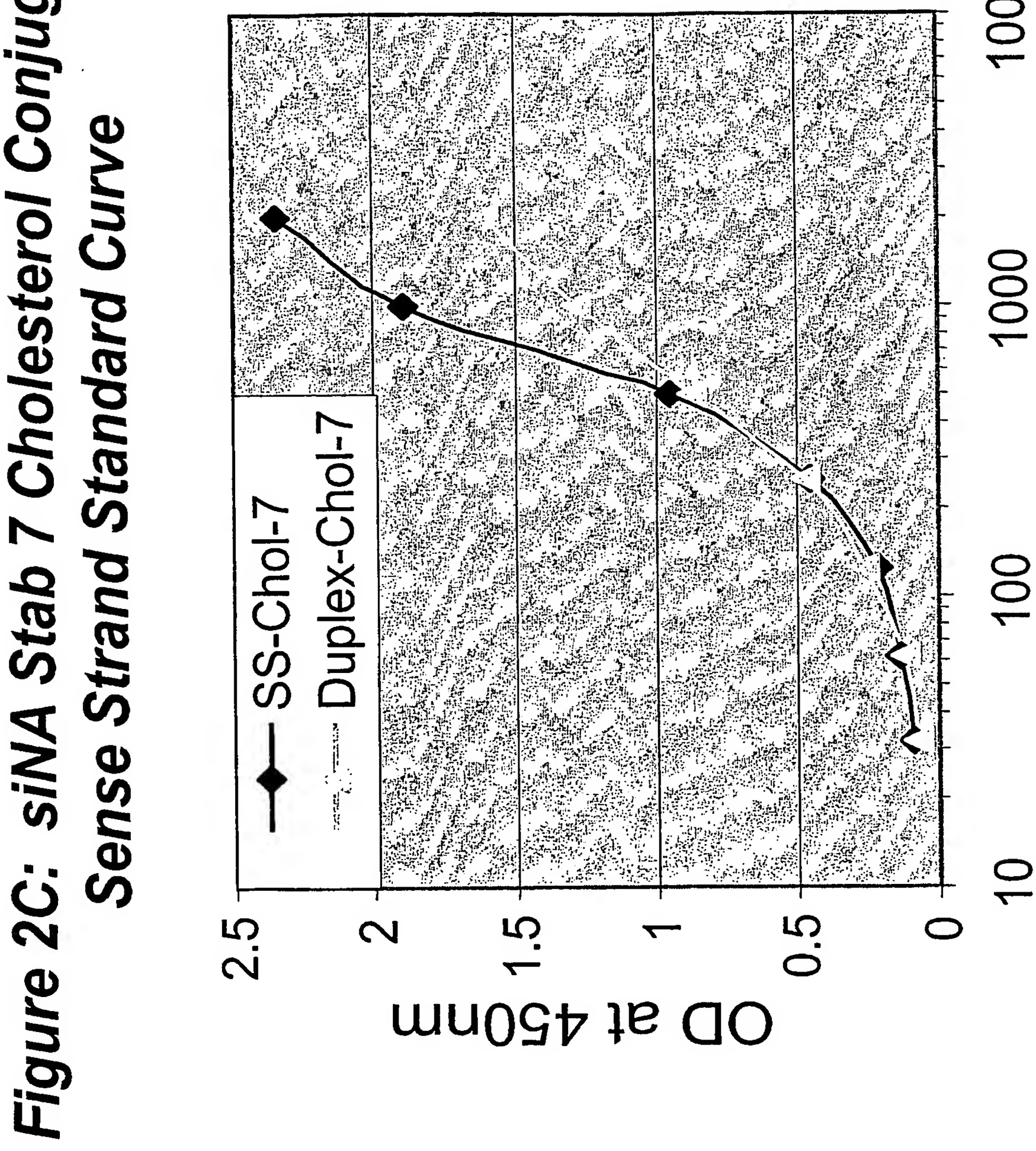


Figure 2C: siNA Stab 7 Cholesterol Conjugate
Sense Strand Standard Curve

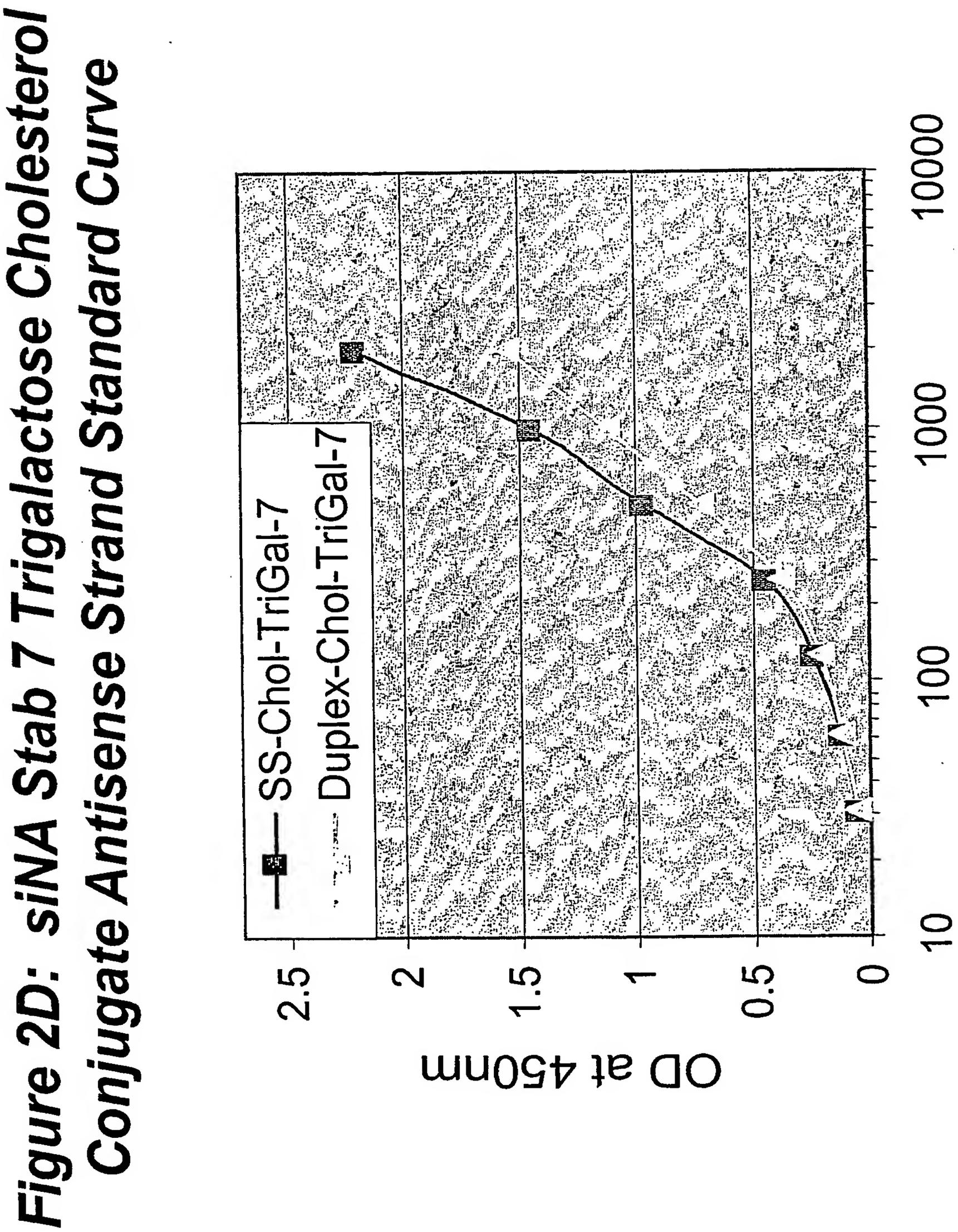


Figure 2D: siNA Stab 7 Trigalactose Chol Conjugate Antisense Strand Standard Curve

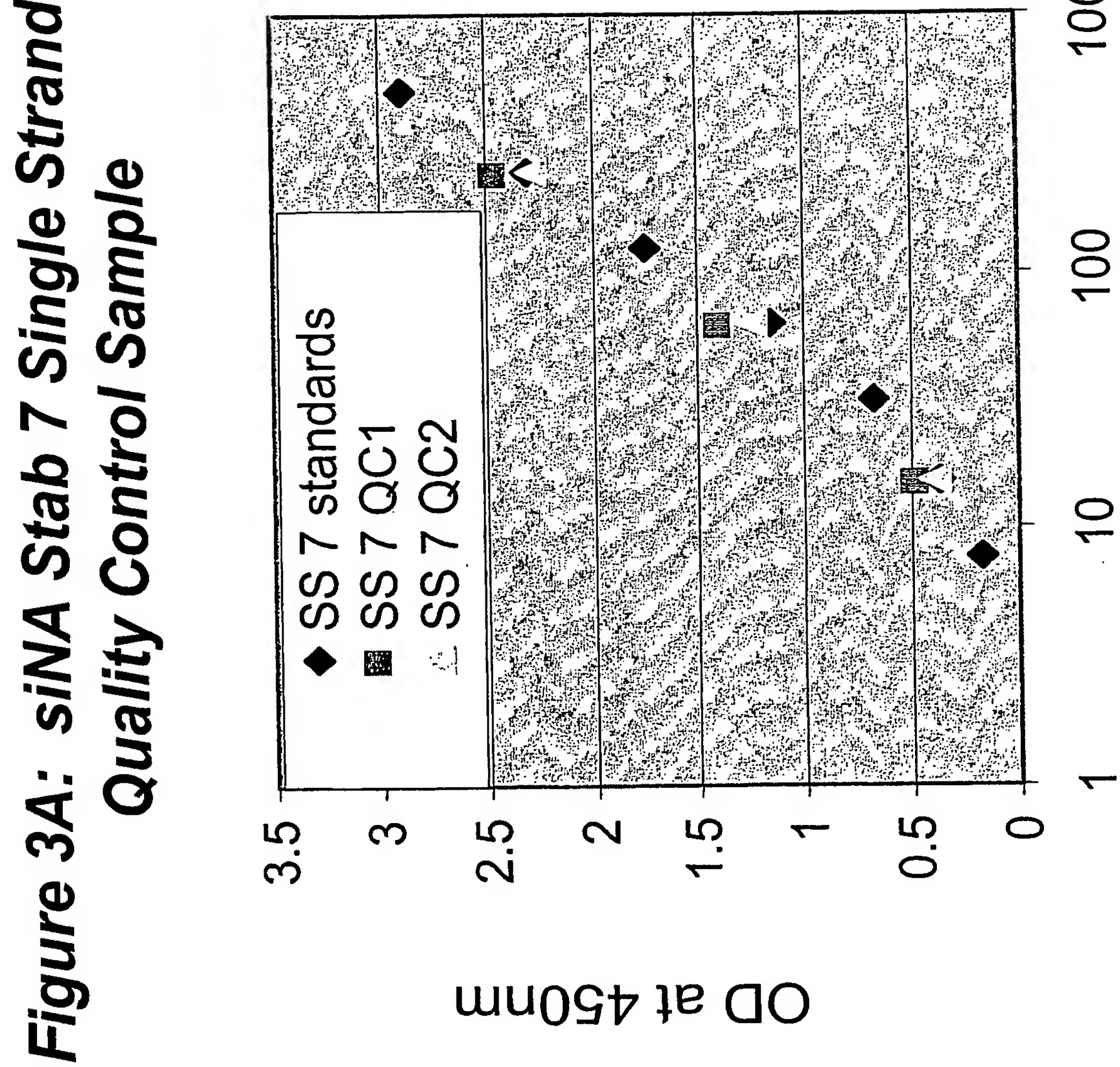


Figure 3A: siNA Stab 7 Single Stranded Quality Control Samples

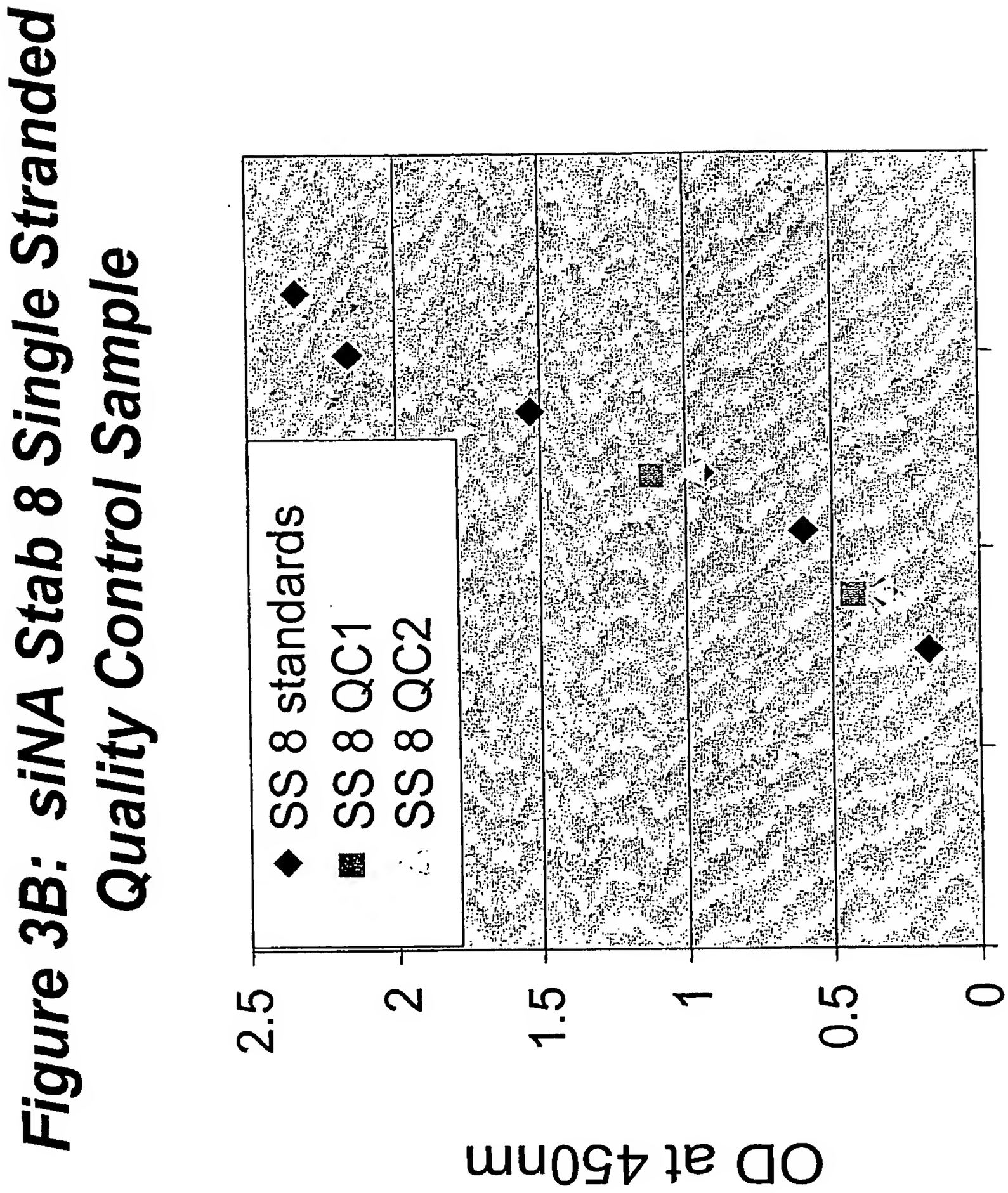


Figure 3B: siNA Stab 8 Single Strand Quality Control Sample

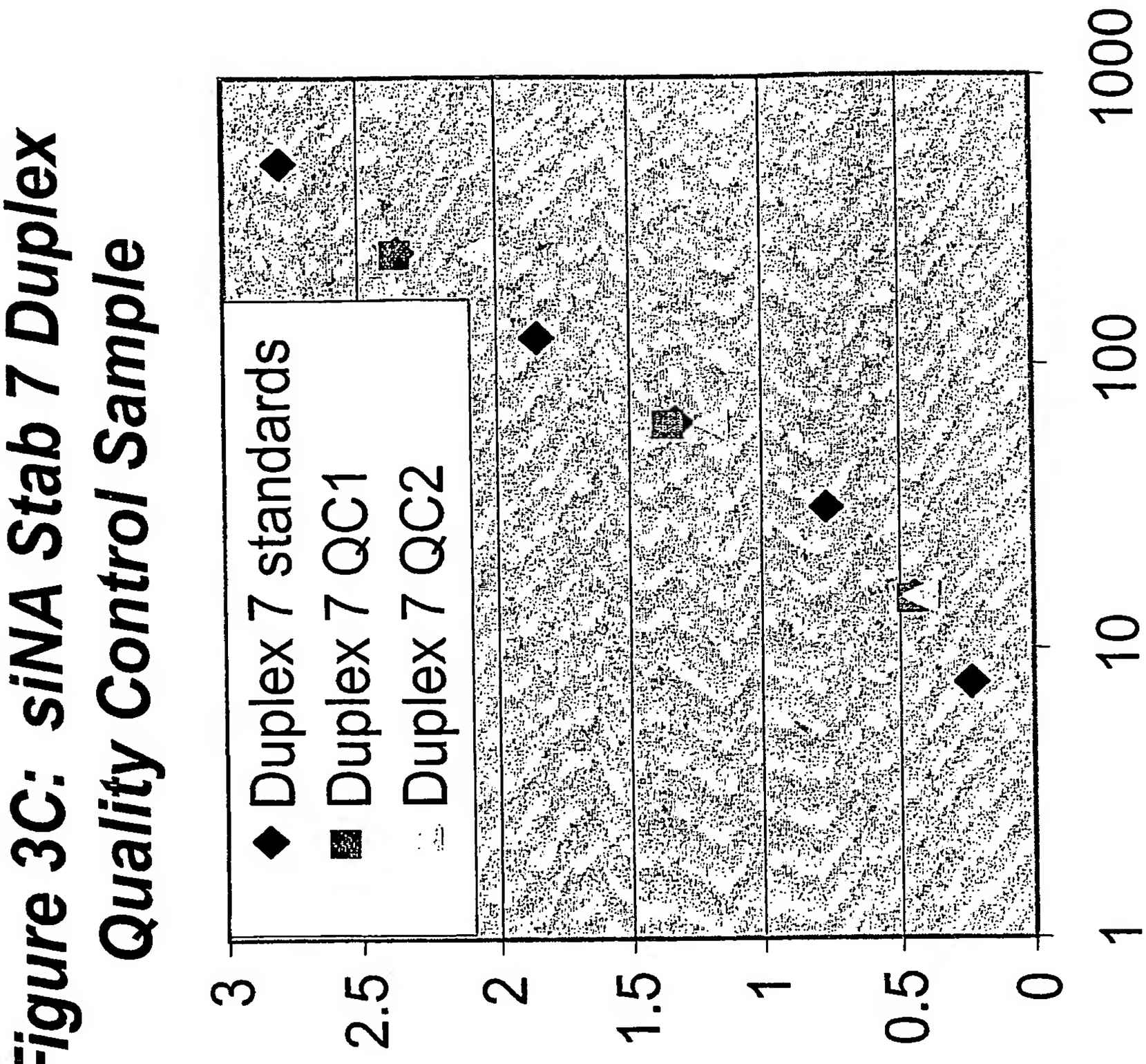


Figure 3C: siNA Stab 7 Duplex
Quality Control Samples
Duplex 7 standards
Duplex 7 QC1
Duplex 7 QC2
OD at 450nm
3
2.5
2
1.5
1
0.5
0
1
10
100
1000
fmols/ml

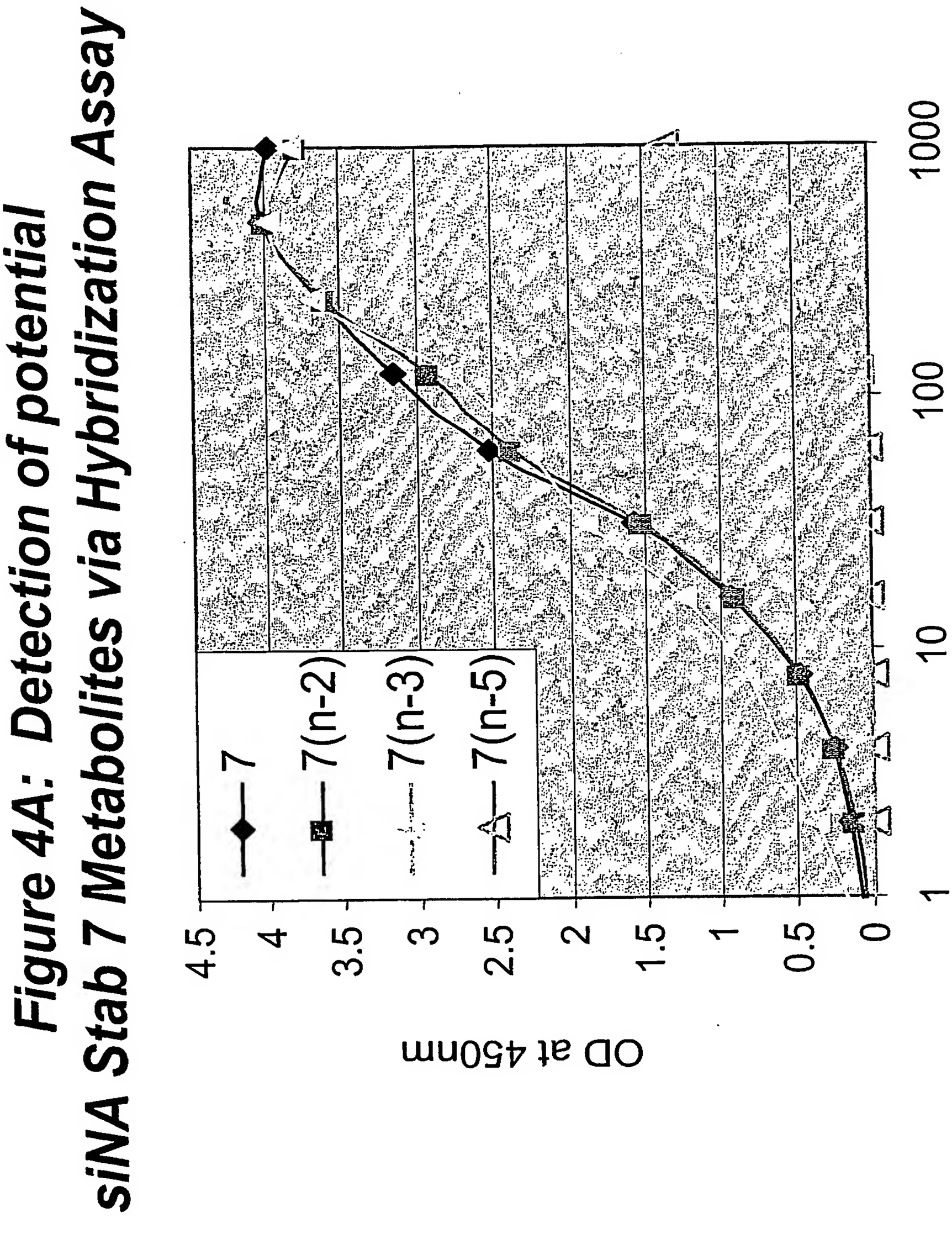


Figure 4A: Detection of potential
siNA Stab 7 Metabolites via Hybridization Assay
1000
7
7(n-2)
7(n-3)
7(n-5)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
OD at 450nm
1
10
100
fmoles / ml

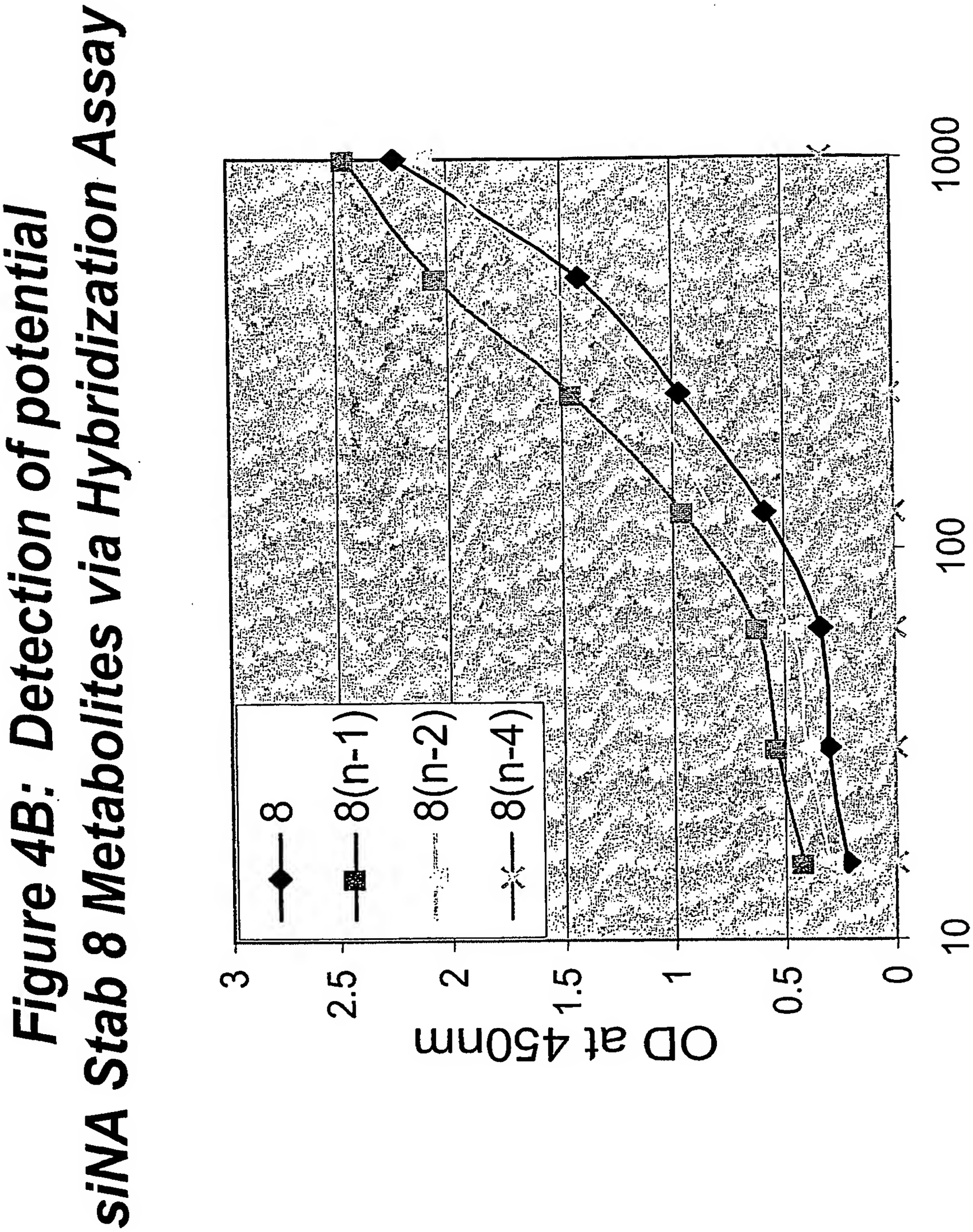


Figure 4B: Detection of potential siNA Stab 8 Metabolites via Hybridization Assay

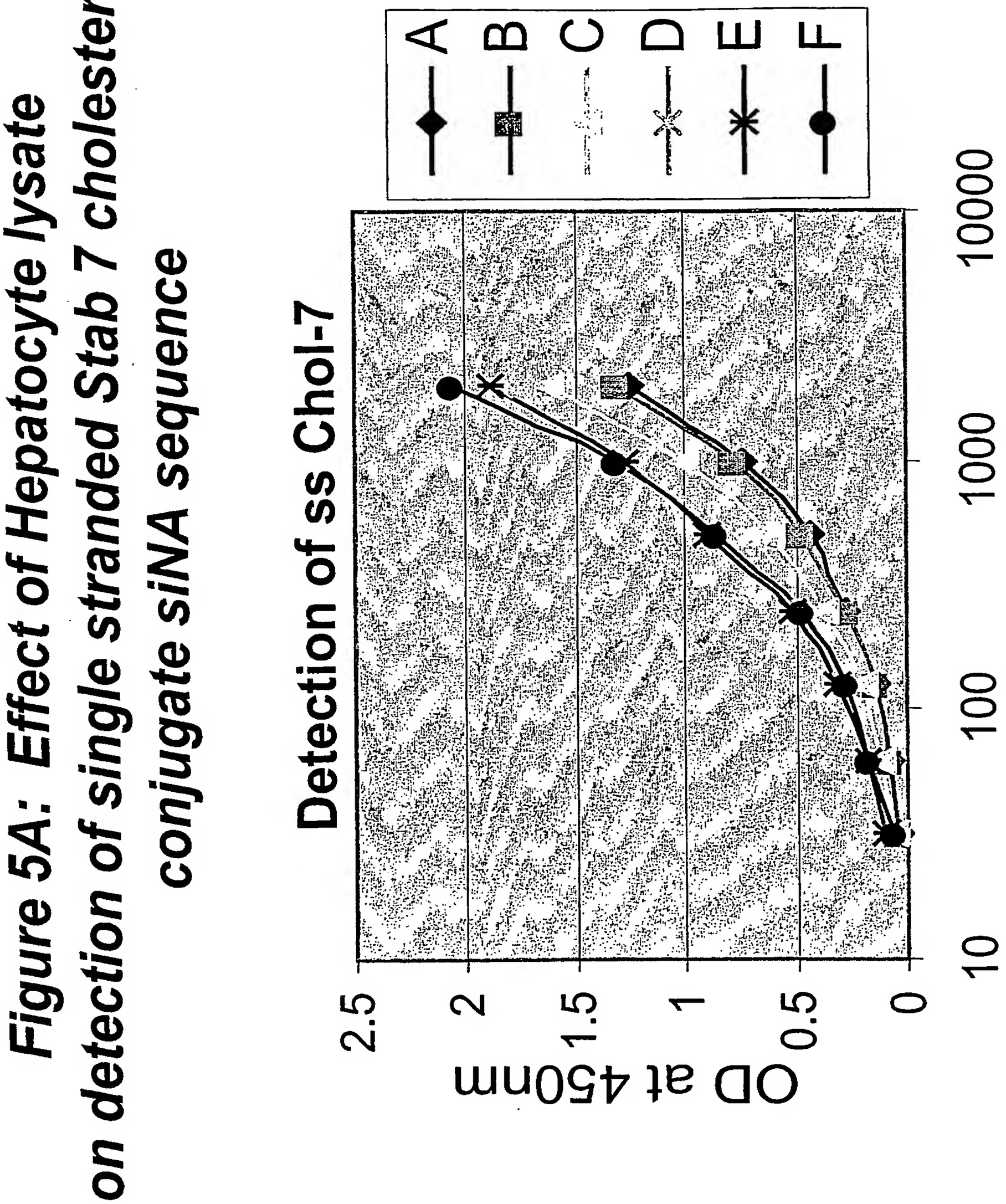


Figure 5A: Effect of Hepatocyte lysate on detection of single stranded Stab 7 cholesterol conjugate siNA sequence

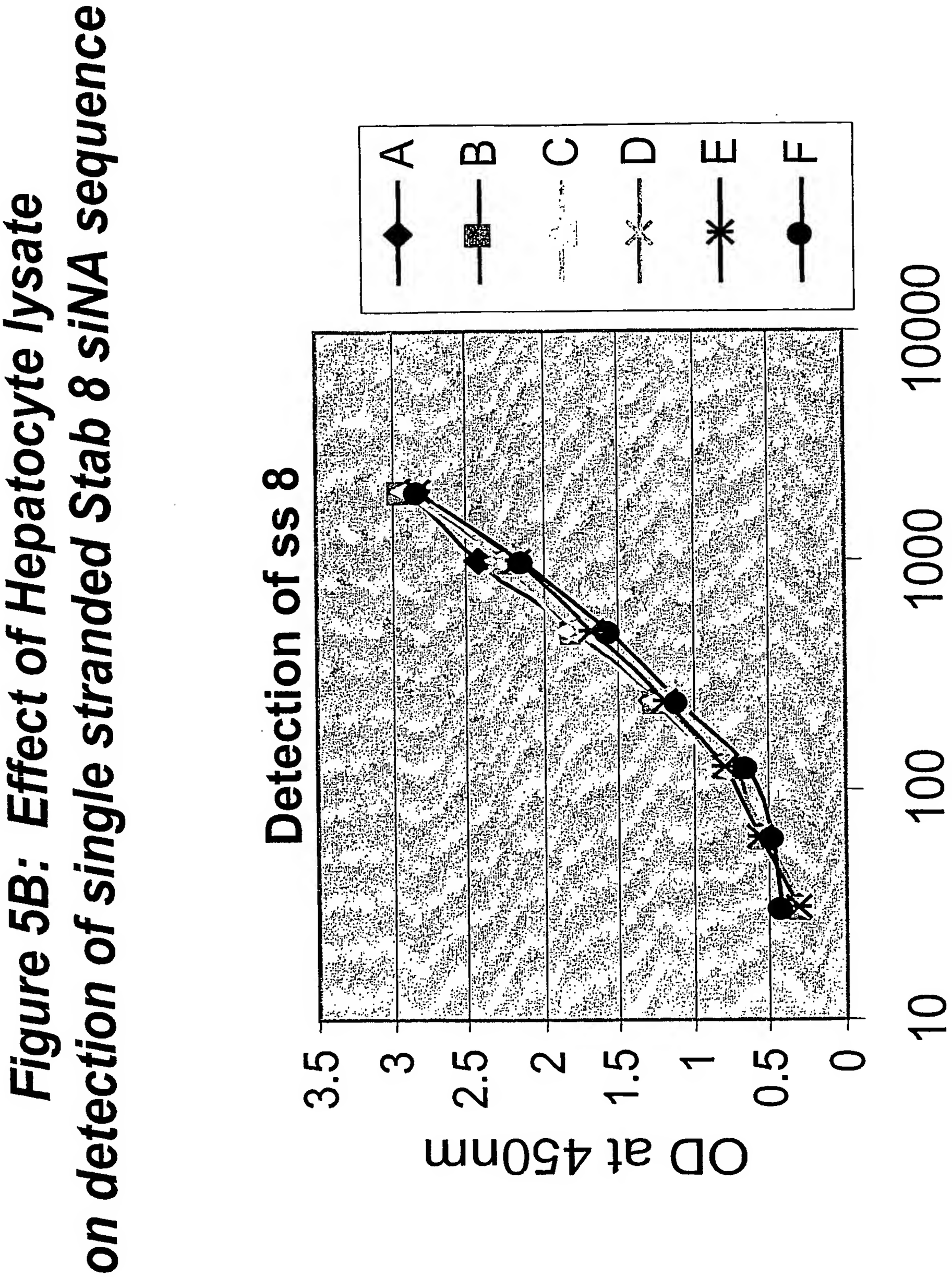


Figure 5B: Effect of Hepatocyte lysate on detection of single stranded Stab 8 siRNA sequence

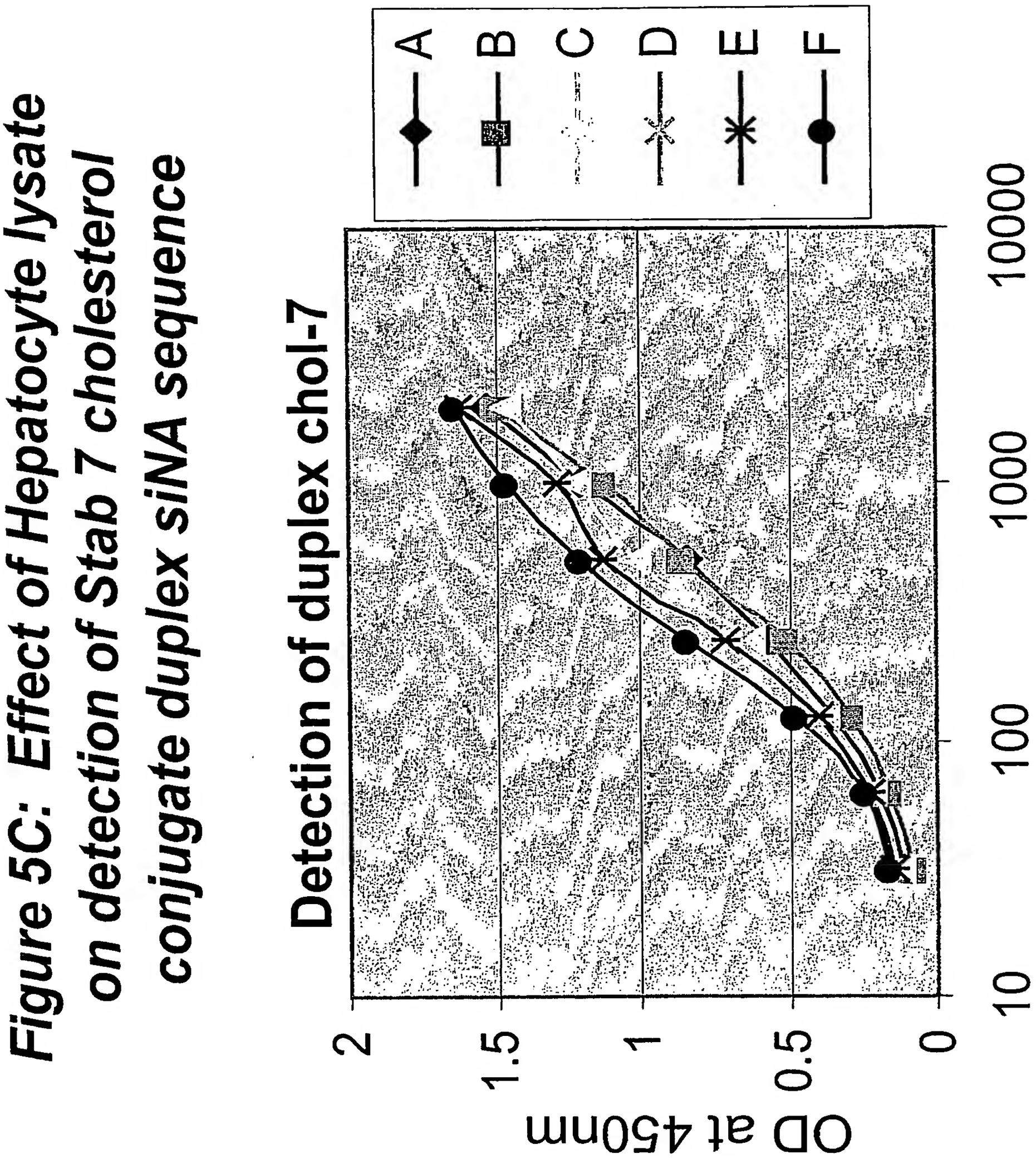


Figure 5C: Effect of Hepatocyte lysate on detection of Stab 7 cholesterol conjugate duplex siNA sequence

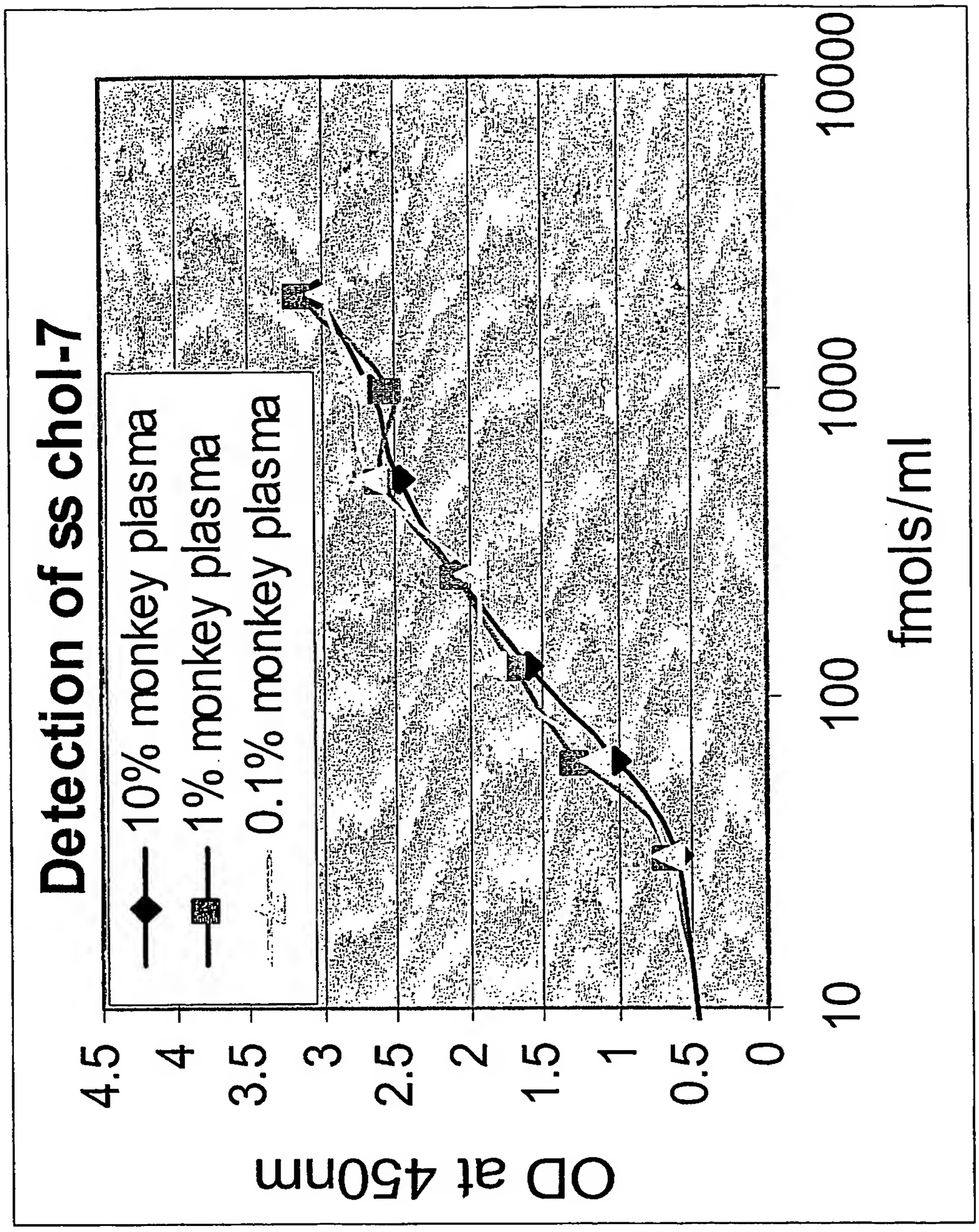


Figure 6A: Effect of monkey plasma on detection of single stranded Stab 7 chol conjugate siNA sequence

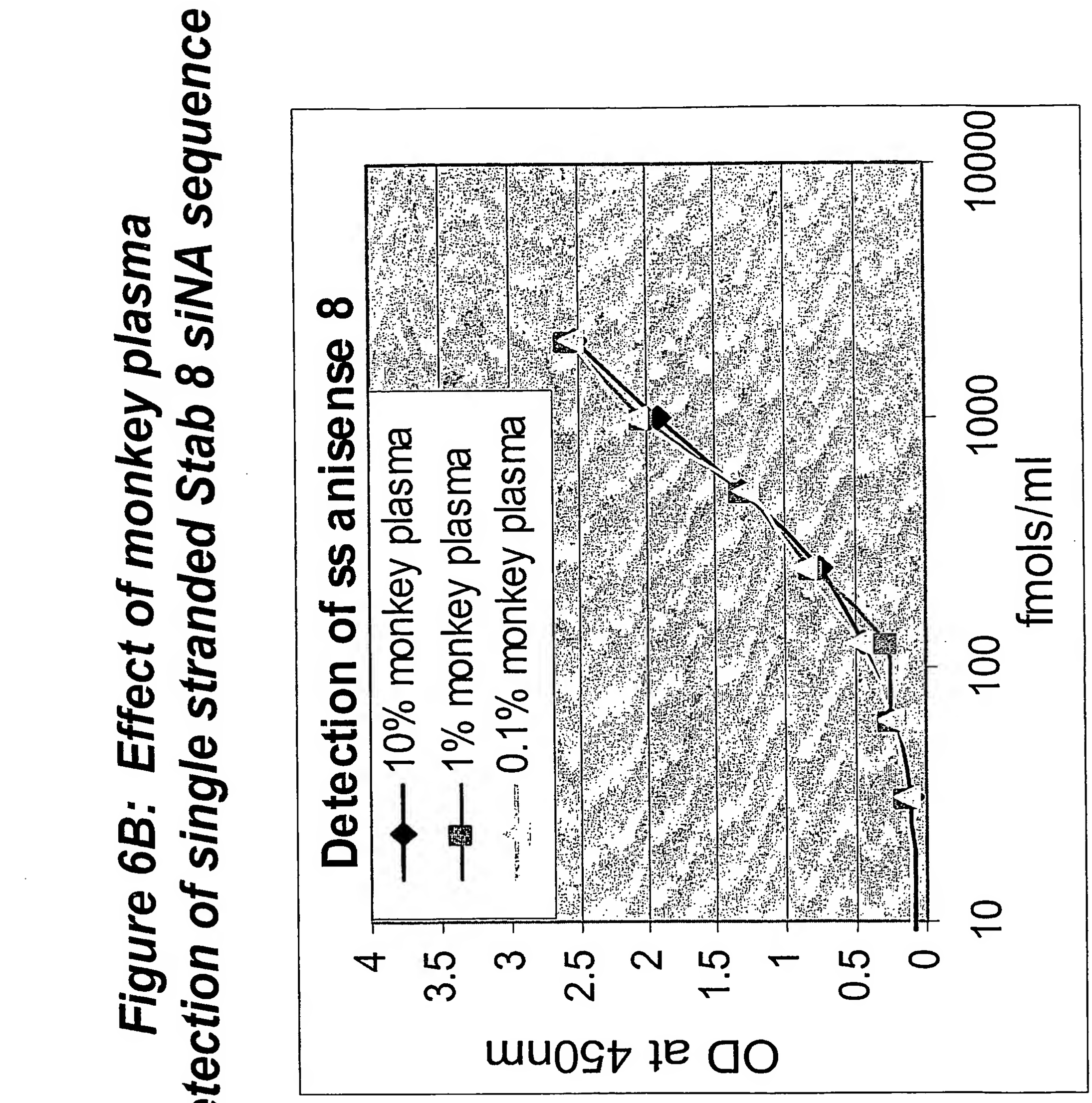


Figure 6B: Effect of monkey plasma on detection of single stranded Stab 8 siNA sequence

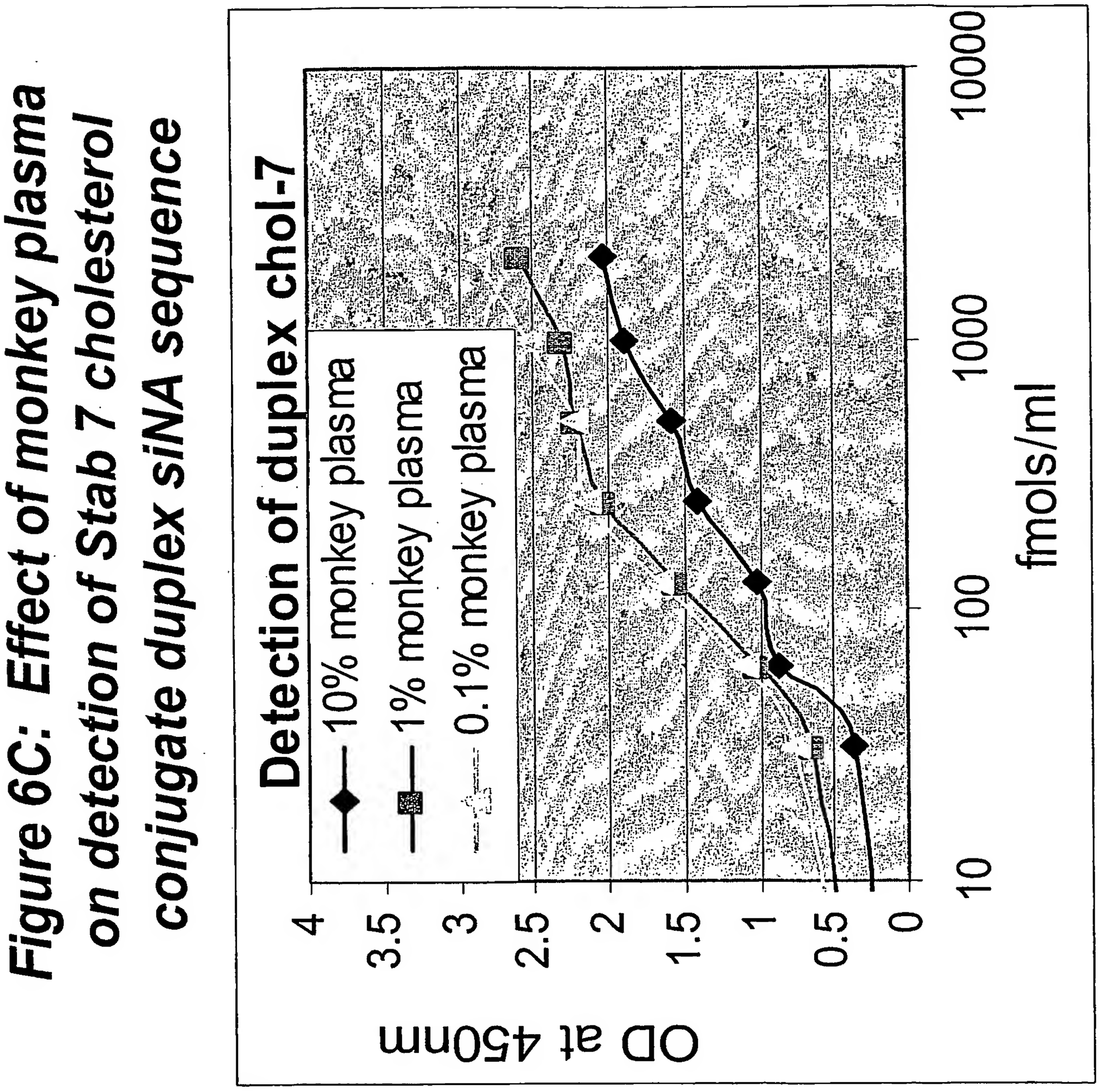


Figure 6C: Effect of monkey plasma on detection of Stab 7 cholesterol conjugate duplex siNA sequence

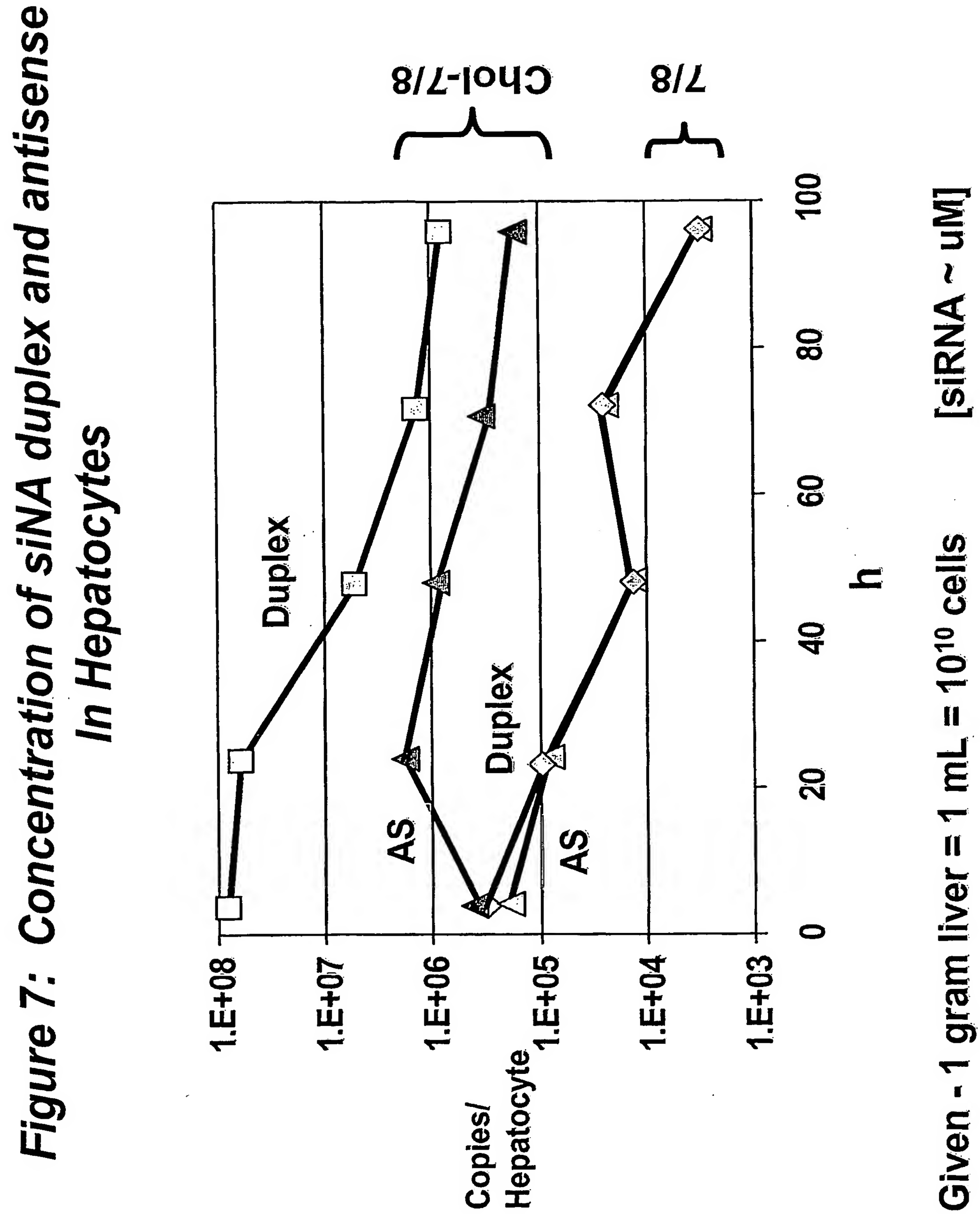


Figure 7: Concentration of siNA duplex and antisense In Hepatocytes

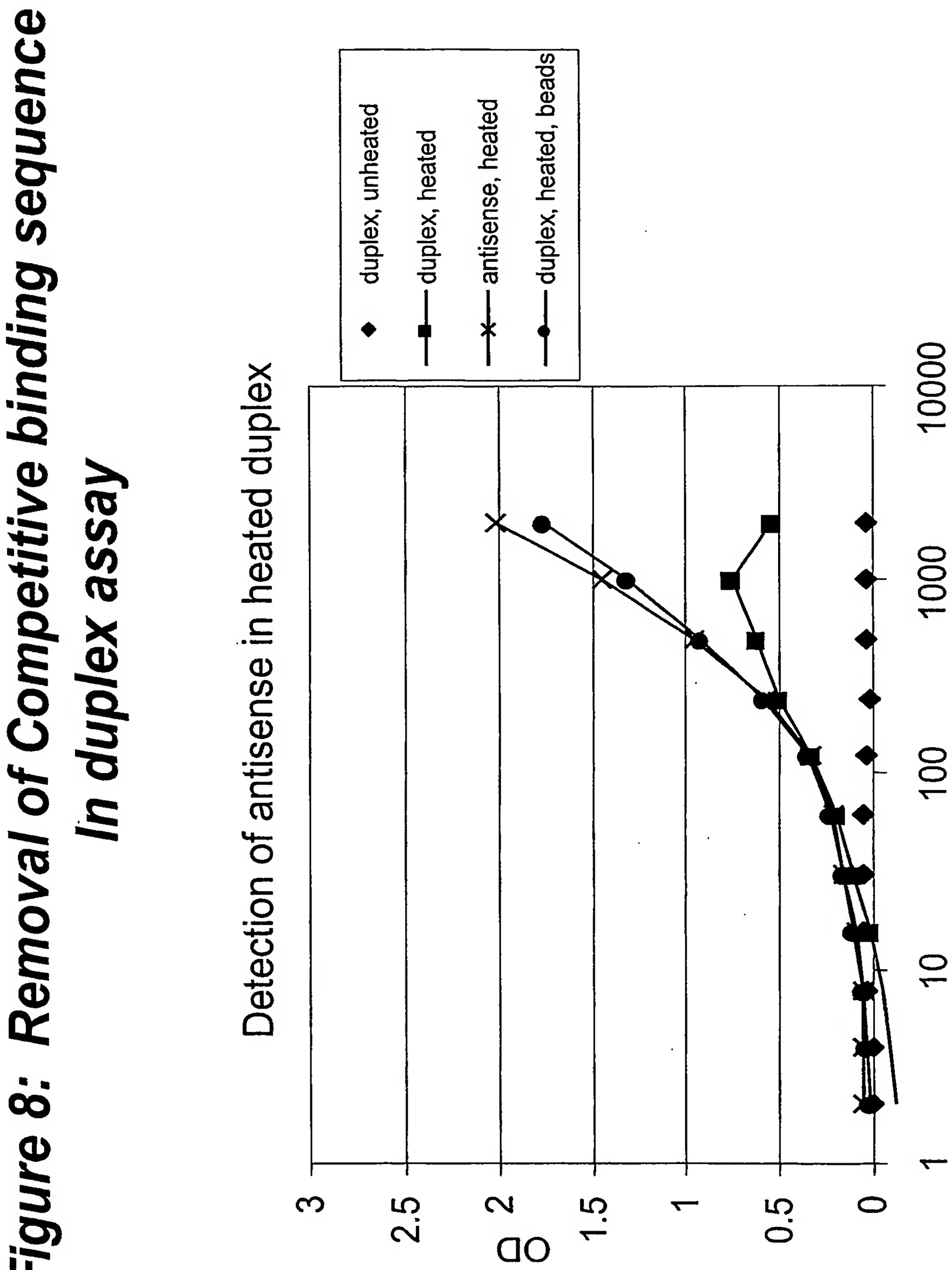

Removal of Competitive binding sequence
In duplex assay
Detection of antisense in heated duplex assay
duplex, unheated
duplex, heated
antisense, heated
duplex, heated, beads
OD
3
2.5
2
1.5
1
0.5
0
10
100
1000
10000
[antisense], fmoles/ml

Figure 8: Re

Figure 9: Application of Hybridization Assay to siNA molecules having identical sequence with differing chemical modifications

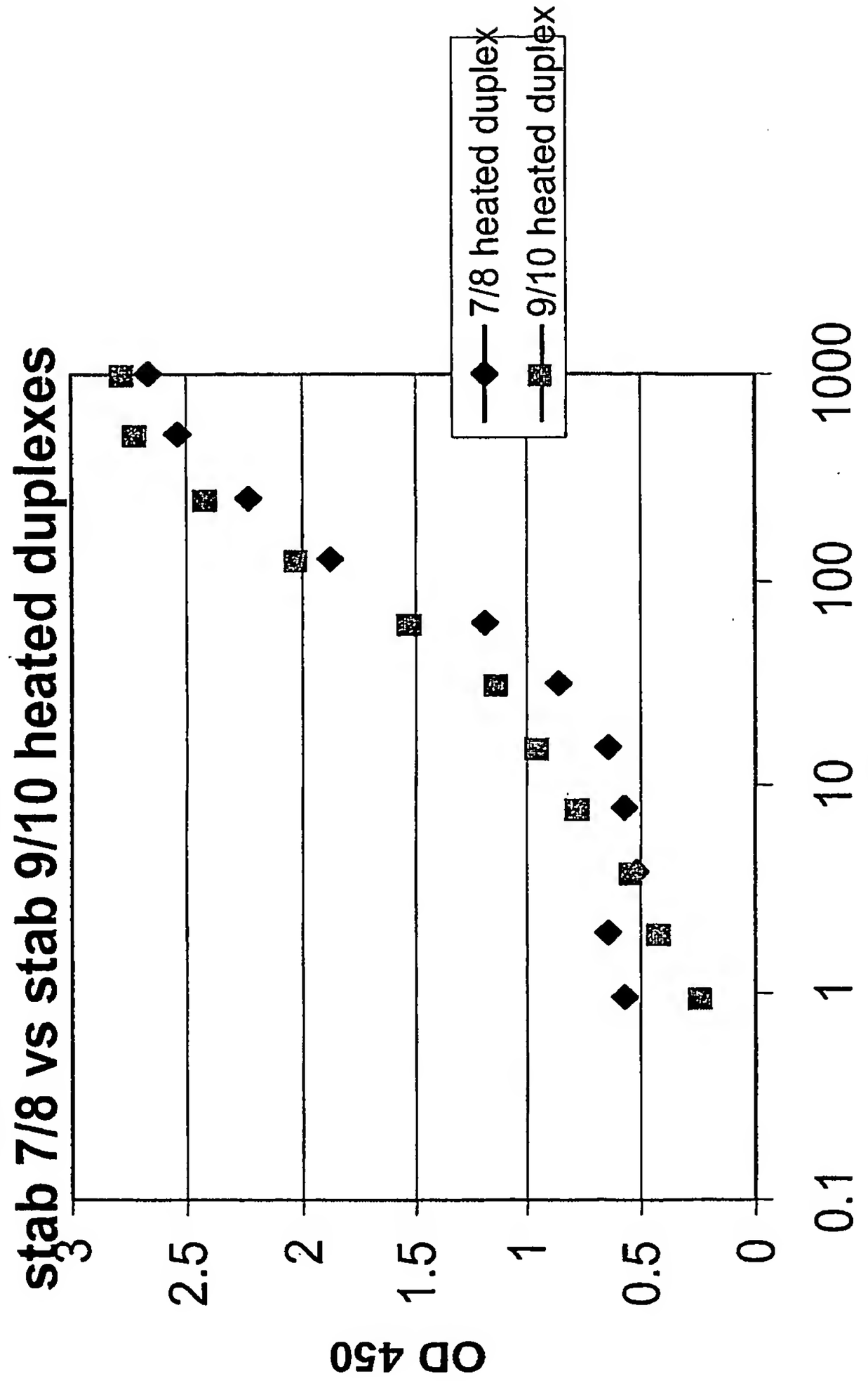
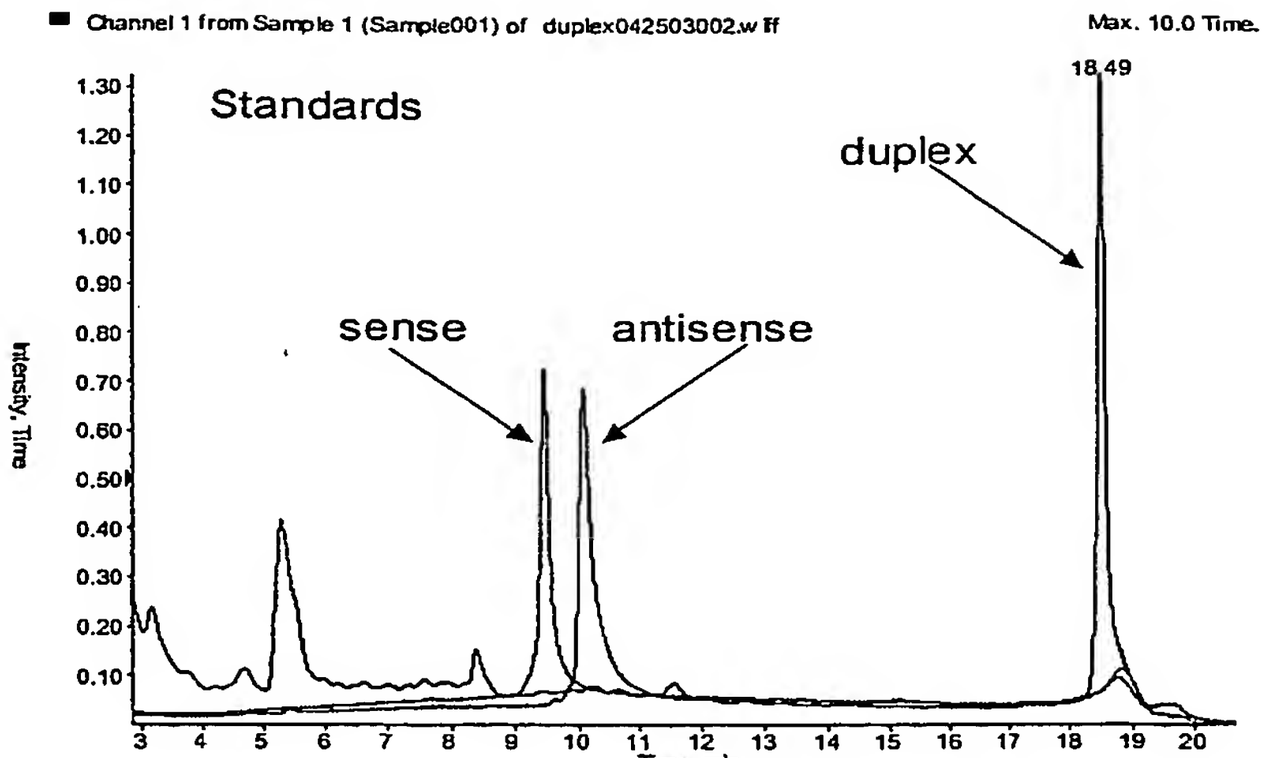
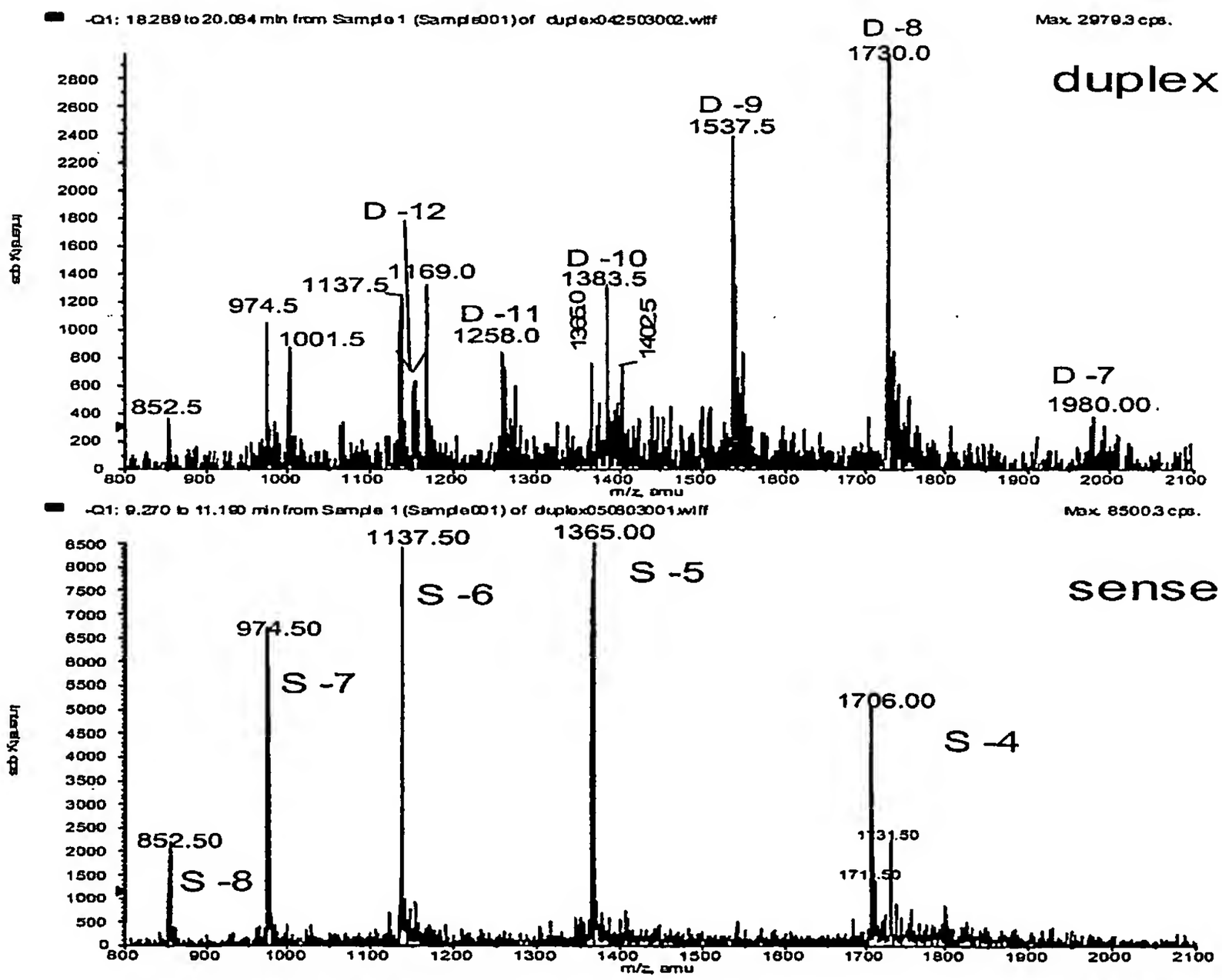


Figure 10

A


Channel 1 from Sample 1 (Sample001) of duplex042503002.w lff
Max. 10.0 Time.
18.49
Standards
duplex
sense
antisense
Intensity, Time
Time, min

B


-Q1: 18.289 to 20.084 min from Sample 1 (Sample001) of duplex042503002.wiff
Max. 2979.3 cps.
D -8
1730.0
duplex
D -9
1537.5
D -12
1169.0
1137.5
974.5
1001.5
D -11
1258.0
D -10
1383.5
1365.0
1402.5
852.5
D -7
1980.00
m/z, amu
-Q1: 9.270 to 11.190 min from Sample 1 (Sample001) of duplex050803001.wiff
Max. 8500.3 cps.
1137.50
S -6
1365.00
S -5
sense
974.50
S -7
1706.00
S -4
852.50
S -8
1731.50
1711.50
m/z, amu

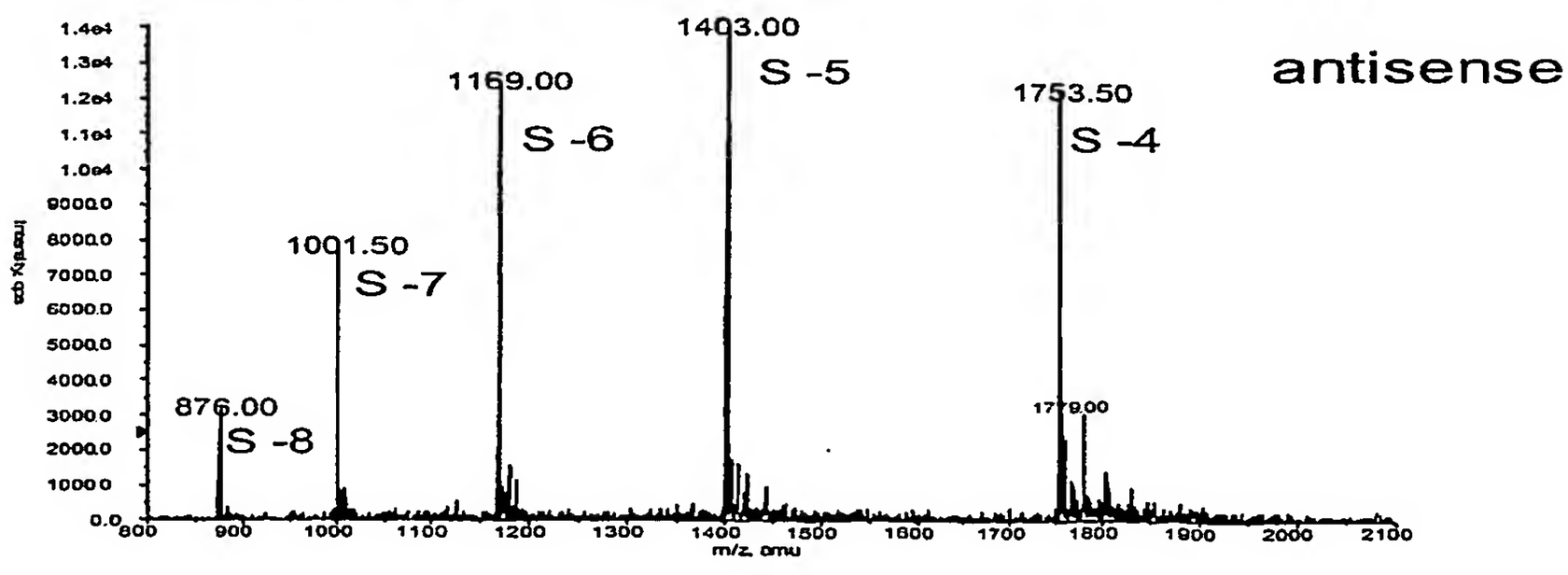
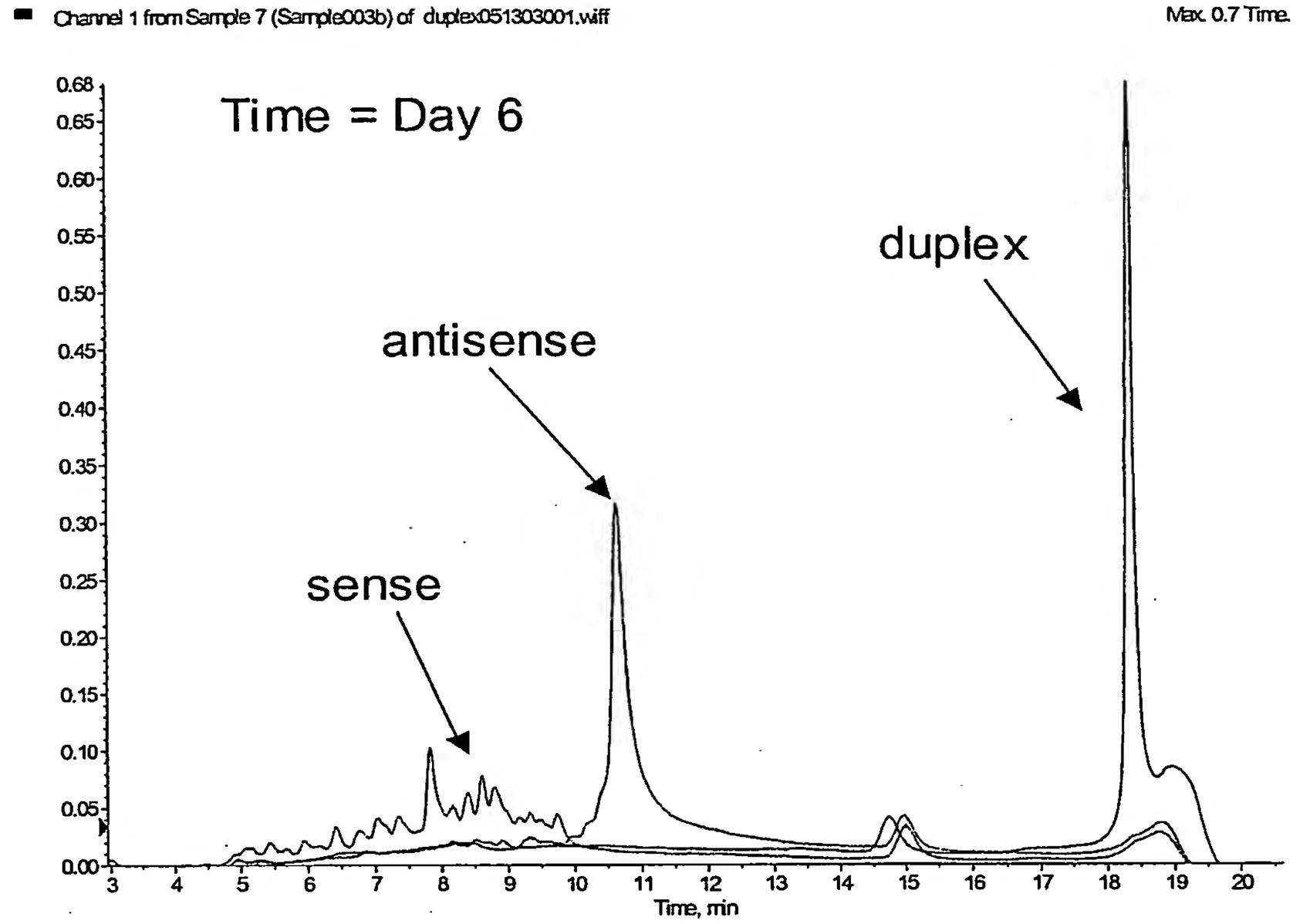

-Q1: 10.105 to 11.884 min from Sample 2 (Sample002) of duplex050803001.wiff
Max. 1.4e4 cps.
1403.00
S -5
1169.00
S -6
antisense
1753.50
S -4
1001.50
S -7
876.00
S -8
1779.00
m/z, amu

Figure 11

A


Channel 1 from Sample 7 (Sample003b) of duplex051303001.wiff
Max. 0.7 Time.
Time = Day 6
antisense
duplex
sense
Time, min

B

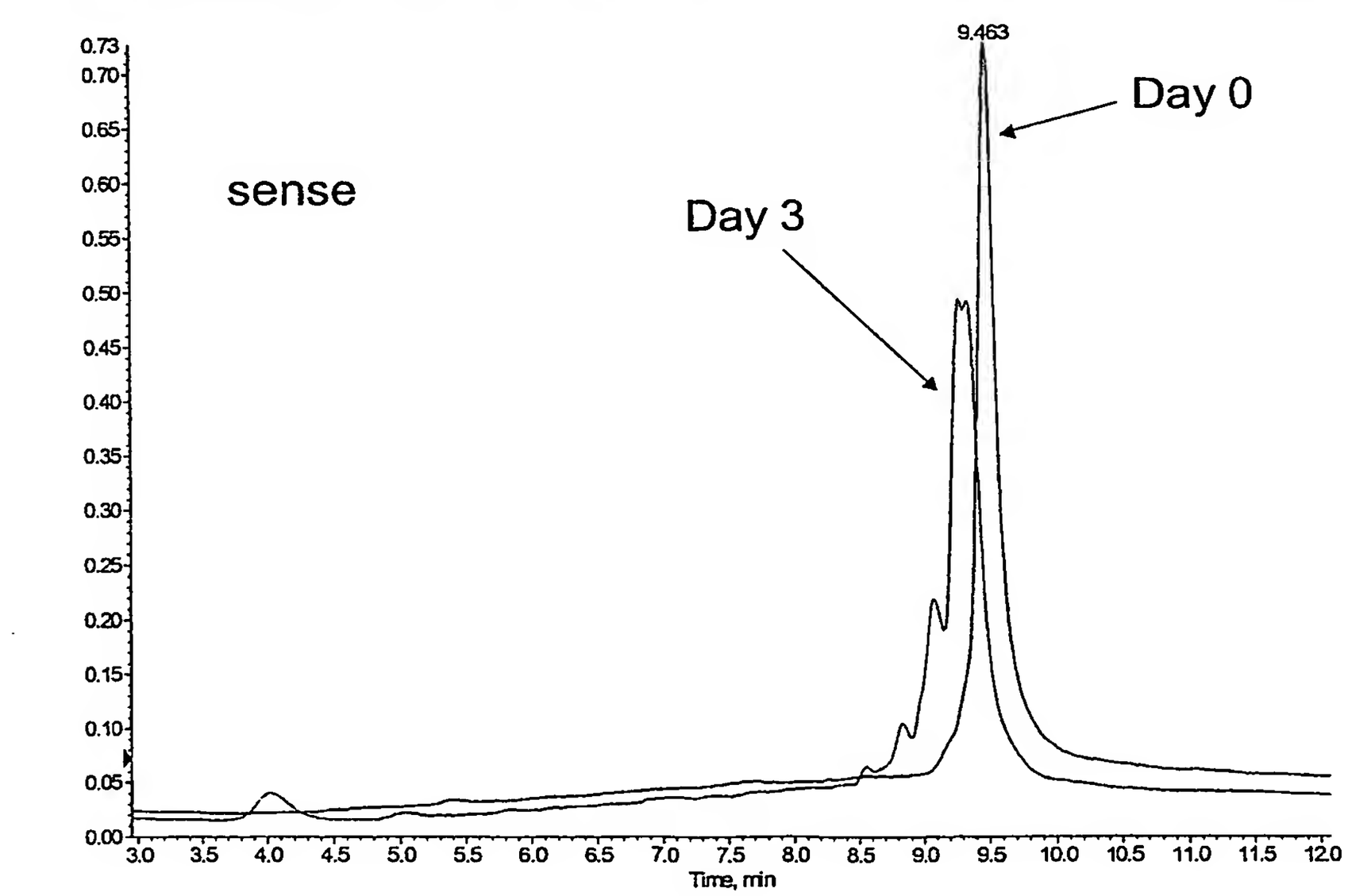

A/D Converter Channel 1 from Sample 1 (Sample001) of duplex050803001.wiff
Max. 0.7 Time.
9.463
Day 0
sense
Day 3
Time, min

Figure 12

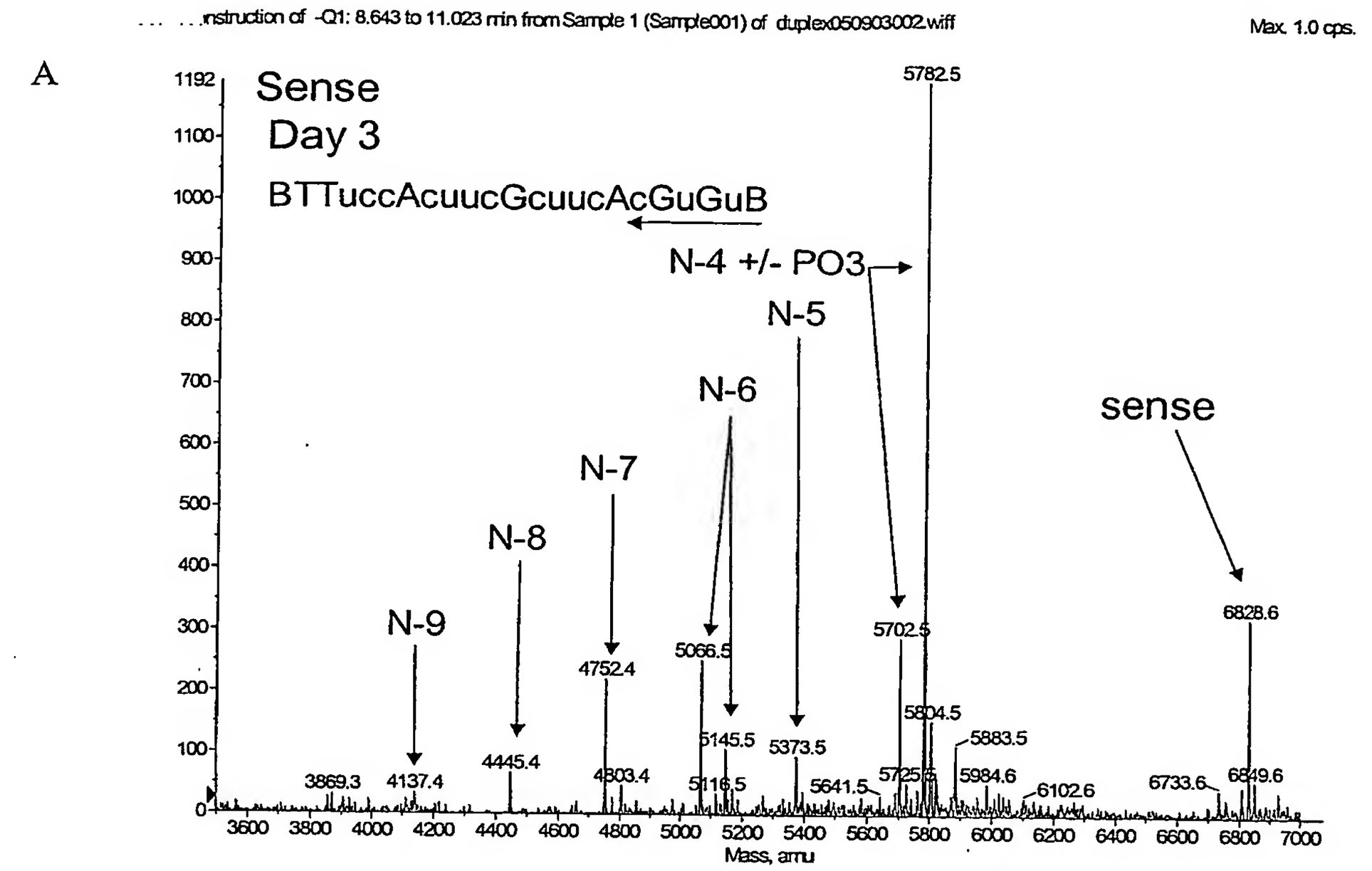
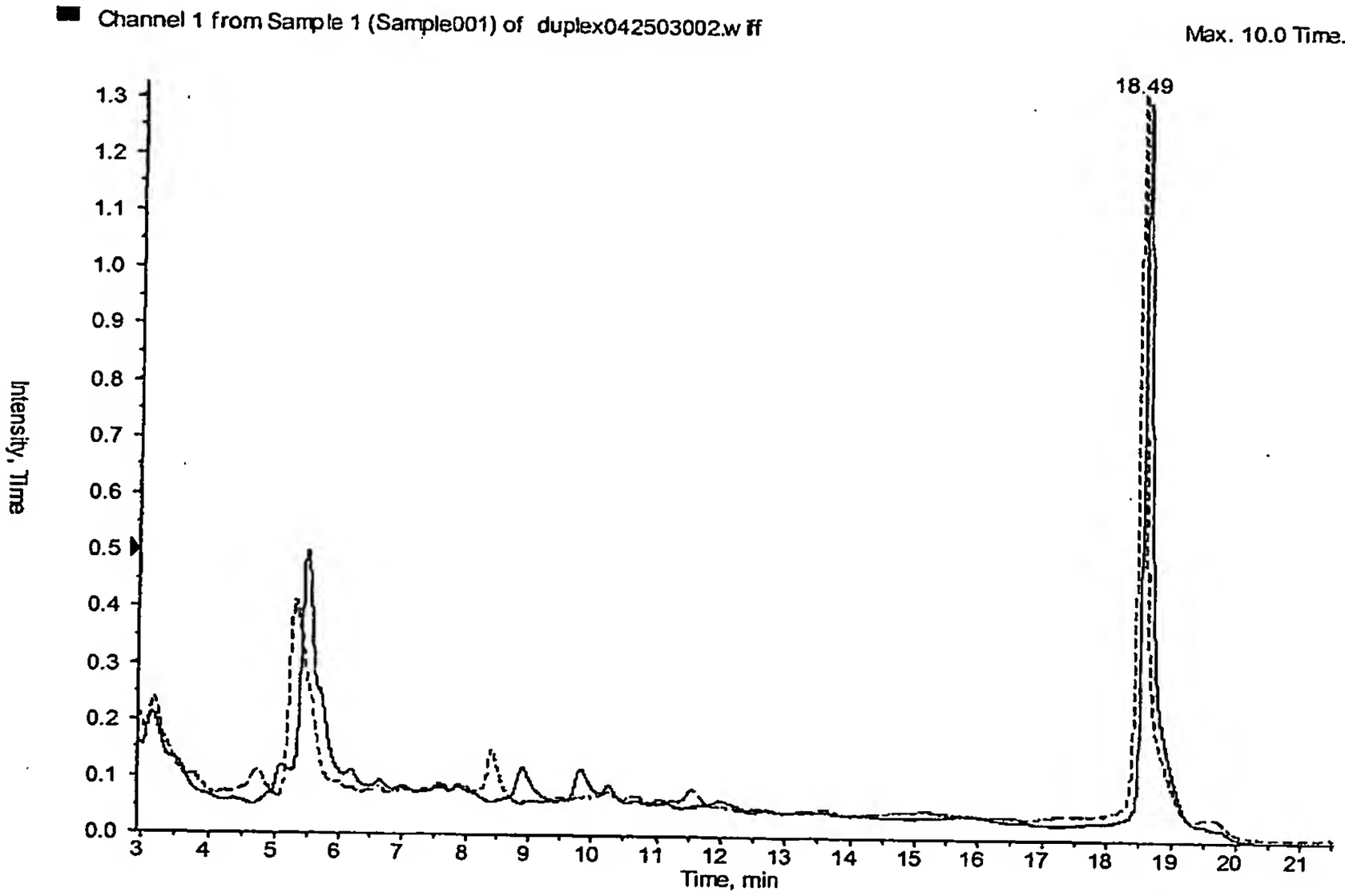

Mass reconstruction of -Q1: 8.643 to 11.023 min from Sample 1 (Sample001) of duplex050903002.wiff
Max. 1.0 cps.
A
Sense
Day 3
BTTuccAcuucGcuucAcGuGuB
N-4 +/- PO3
N-5
N-6
N-7
N-8
N-9
sense
5782.5
6828.6
5702.5
5066.5
4752.4
5804.5
5883.5
5145.5
5373.5
4445.4
3869.3
4137.4
4803.4
5116.5
5641.5
5984.6
6102.6
6733.6
6849.6
Mass, amu
Mass reconstruction of -Q1: 9.520 to 10.981 min from Sample 2 (Sample002) of duplex051603001.wiff
Max. 1.0 cps.
B
antisense
Day 6
TsTAcAcGuGAAGcGAAGuGGA
N-1 +/- PO3
N-2
N-4
N-5
N-6
N-8
N-9
N-10
7017.6
6755.6
4015.3
5728.5
6395.5
6675.5
5368.4
3655.3
5024.4
4679.4
5406.4
5644.5
5939.5
6627.5
6929.6
7075.6
Mass, amu

Figure 13

A



Mass reconstruction of -Q1: 17.787 to 19.833 min from Sample 15 (Sample005a) of duplex05050..Max. 1836.9 cps.

B

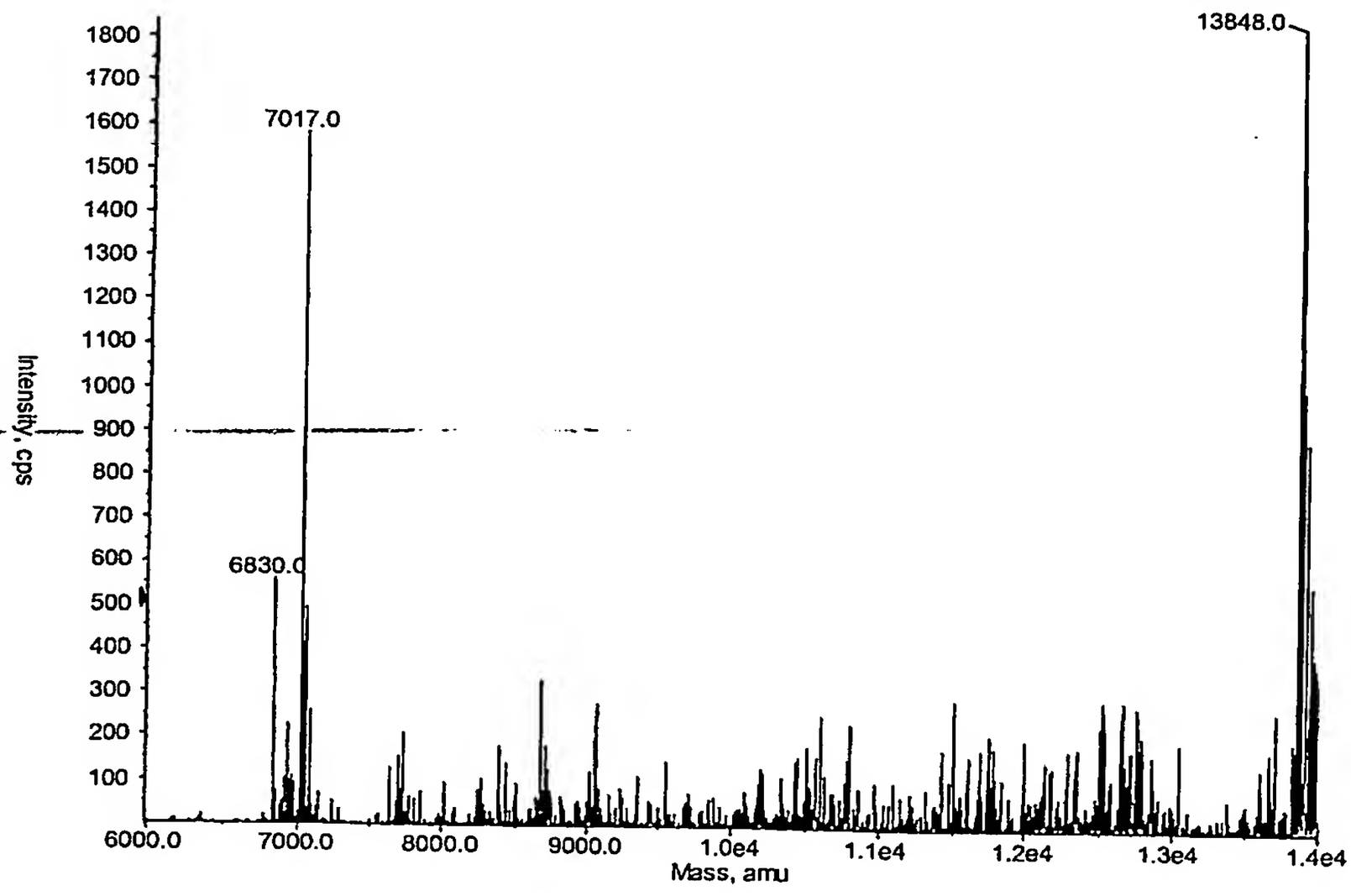


Figure 14

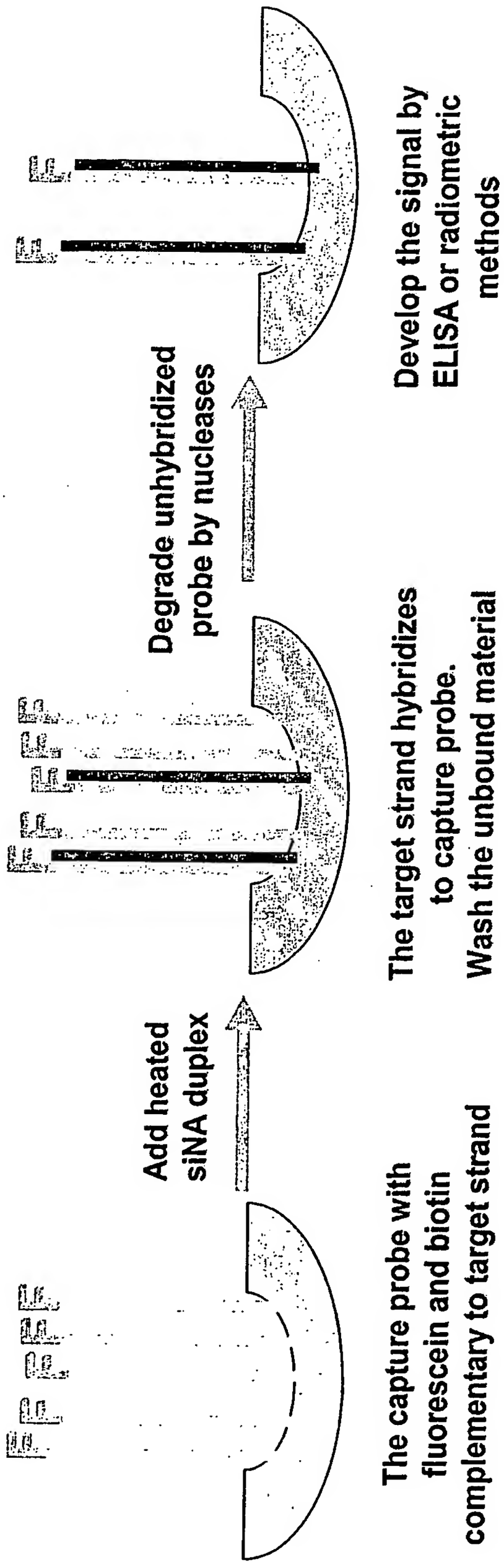
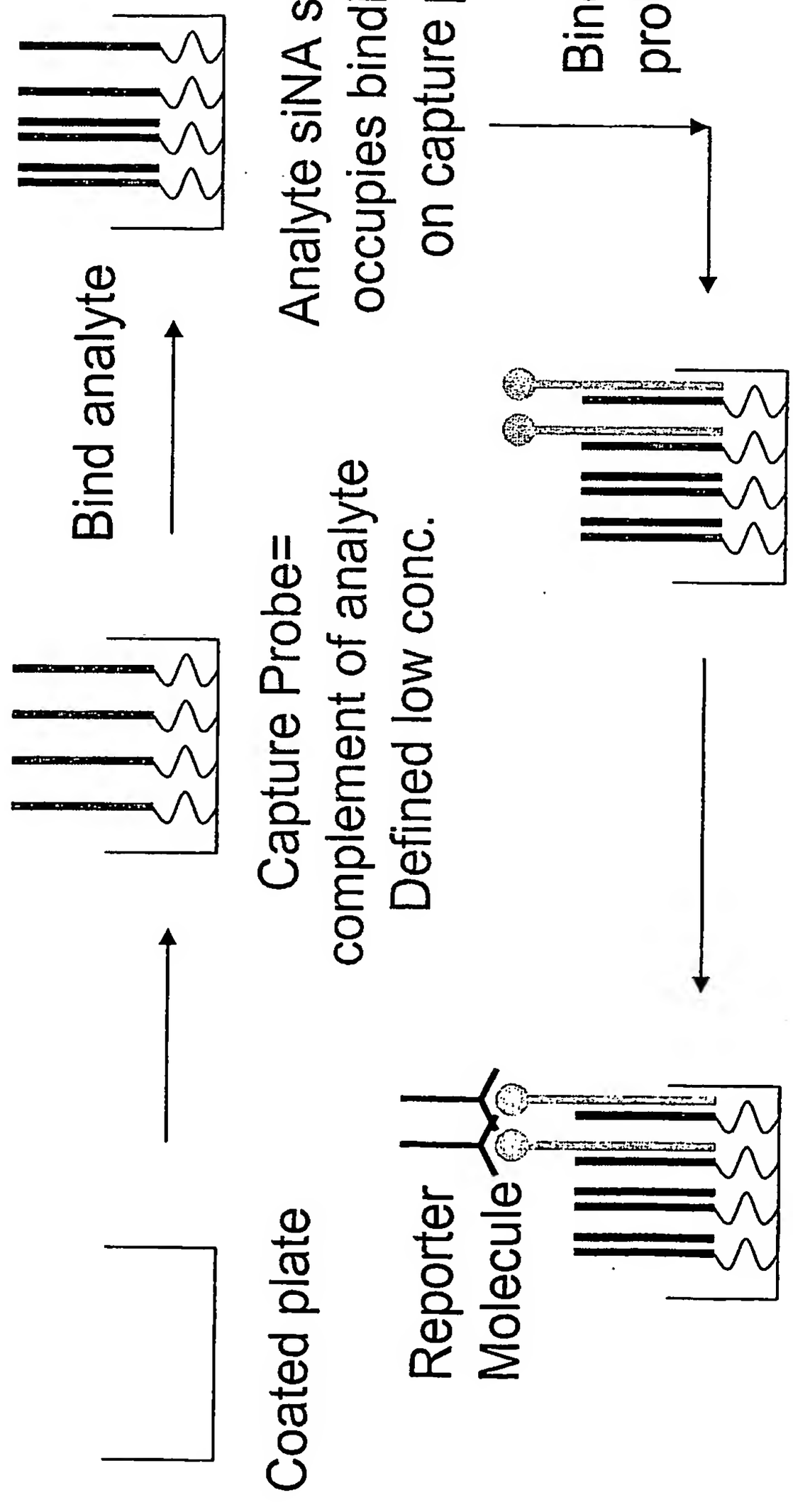

Develop the signal by ELISA or radiometric methods
Degrade unhybridized probe by nuclease
The target strand hybridizes to capture probe. Wash the unbound material
Add heated siNA duplex
The capture probe with fluorescein and biotin complementary to target strand
= target polynucleotide
= Labeled Capture Probe

Figure 15



In this design, binding of the target siRNA (in step 1) prevents binding of a secondary detection probe (in step 2). Therefore, signal is inversely proportional to analyte concentration.